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ERRATA

p. 156. For **"PHOTOSIPHON"** in the title read **"PROTOSIPHON."**

p. 130, 12 lines from bottom. For *"monlhs"* read *"monlhs."*

p. 385, l. 12. Change *"Spathoglossum"* to *"Spatoglossum."*

p. 389, 4 lines from bottom. For *"fragillissima"* read *"fragilissima."*

p. 391, 4 lines from bottom. Change "SCHROEDERI" to SCHRODERI."

p. 393, l. 10. For "SUVERTICILLATA" read "SUBVERTICILLATA."

p. 394, l. 10. For *"Digenia"* read *"Digenea."* 10 lines from bottom. For
"GYMNOGONRUS." read "GYMNOGONGRUS."

p. 430, 10 lines from bottom. For *"albapilosa"* read *"albopilosa."*

PYTHIUM CROWN ROT OF RHUBARB[1]

John T. Middleton

INTRODUCTION

Rhubarb, *Rheum Rhaponticum* L., is a popular early spring vegetable grown throughout most of the United States, although more commonly in the northern than in the southern latitudes The two principal districts in which rhubarb is produced commercially are New Jersey and California.

In California most of the commercial rhubarb is grown in the San Francisco Bay area, chiefly in Alameda County, and in the immediate vicinity of Los Angeles. Rhubarb is usually planted in relatively small acreages, varying in size from one to forty acres In northern California it is generally grown on heavy silt-loam soils, without irrigation; in southern California it is most frequently planted on relatively light, sandy, irrigated soil. Rhubarb may be harvested throughout the year in California, but the bulk of the crop is taken in the early spring, both for local use and for eastern shipment.

Crown rot of rhubarb was observed in March, 1936, in the San Francisco Bay area,[2] and in February, 1940, in the vicinity of Los Angeles. A number of plants in several fields in each of the two localities were affected, demonstrating that the disease occurs regardless of soil type and irrigation practice Isolations from affected material yielded pure cultures of several species of *Pythium*.

REVIEW OF THE LITERATURE

A number of rots are reported as affecting the root, crown, and stem of the rhubarb plant with symptoms similar to those associated with the presence of *Pythium* spp. but due to other organisms. The one most likely to be confused with the rot caused by *Pythium* is that due to species of *Phytophthora*

Beach (1921) described a wilt of rhubarb in which the leaves wilted, turned yellow, and collapsed, and the crown and roots became discolored and rotten; an unidentified species of *Phytophthora* was isolated from the diseased material Beach (1922) later gave a more detailed description of the disease and identified the fungus as *P. cactorum* (L. & C.) Schroet.; several additional reports on the disease were also made (1924, 1925, 1926). Godfrey

[2] Observation by C. M. Tompkins.

(1923) described a disease similar to that given by Beach, but attributed it to *P. parasitica* Dast. var. *rhei* Godfrey, a new variety which Tucker (1931) would abandon, since the fungus conforms with the limits of the species in the broader sense. Infection of rhubarb with *P. parasitica* has been reported in the United States by Tucker (1933a, 1933b, 1935) and by Ramsey and Wiant (1944); in the Union of South Africa by Doidge (1924) and Wager (1931, 1935, 1940); in Rhodesia by Hopkins (1931); it has also been reported in New South Wales (1943). Tucker (1933a) indicates that one of the unidentified species of *Phytophthora* from rhubarb in Illinois, described by Beach, fits the description of *P. parasitica*, though no material is available for study. Barrett (Merrill 1927) reported a decay of roots, crown, and basal portion of the petioles of rhubarb, from which a fungus resembling *Pythiacystis* (syn. *Phytophthora*) was secured.

A *Pythium* sp. was first reported from rhubarb in the United States by Drechsler (1930), who described the fungus as *P. anandrum* Drechs. and stated that it was isolated "from softened underground bud of *Rheum rhaponticum* L. collected near Brentwood, Md . ." Drechsler did not describe the symptoms of the disease, nor did he describe the pathogenicity of the fungus to rhubarb.

Wager (1931) reported the isolation of *Pythium ultimum* Trow, together with *Phytophthora parasitica*, from rotted rhubarb in the Union of South Africa. He established the pathogenicity of *Pythium ultimum* to rhubarb by artificially inoculating injured rhubarb seedlings grown in sterile soil. The fungus was unable to infect uninjured plants. Infected plants very often recovered. Symptoms of the disease caused by *P. ultimum* were similar to those produced by *Phytophthora parasitica*. Wager (1932, 1940) later published further reports on the occurrence of these two fungi on rhubarb.

Middleton (1941, 1943) recorded the occurrence of *Pythium anandrum*, *P. oligandrum* Drechs., and *P. ultimum* from rhubarb grown in California. Ramsey and Wiant (1944) report that *P. ultimum* and other species of *Pythium* are able to infect rhubarb.

SYMPTOMS OF THE DISEASE

Rhubarb plants infected with *Pythium* crown rot usually wilt; the leaves then turn yellow, and later collapse (fig. 1). The basal portions of the petioles of infected leaves generally show sunken, longitudinally oriented, brown streaks $\frac{1}{4}-\frac{1}{2}$ inch wide, which progress upwards about 2–3 inches. Infected crowns (fig. 2) are soft and watersoaked, with the tissues somewhat flaccid, and brown in color; the infection ordinarily progresses to the bud scales, and later to the buds, causing a blight of the shoots. Sometimes young buds escape infection despite the diseased crown, only to become infected when just at or above the soil level. Rarely does the disease develop far above

ground level, its development then depending upon a moist atmosphere
Only the roots of some plants are affected, the cortex becoming flaccid and

FIG. 1. A. Rhubarb plant artificially infected with *Pythium* crown rot. B. Healthy
plant. FIG 2 Longitudinal sections (A) through crown of rhubarb plant artificially in-
fected with *Pythium* crown rot, and (B) through crown of healthy plant.

watersoaked; such plants are usually small, with spindly, somewhat chlorotic
leaves and few marketable petioles. Plants with infected roots occasionally
recover, but often die.

Apparently healthy petioles collected from diseased plants frequently decay after packing, the rot consuming the major portion of the remaining stalks; such petioles are usually taken from plants with shoots that are more or less healthy, but with infected bud scales

THE CAUSAL ORGANISMS

Isolation. Isolation of the fungi concerned was made in the usual manner The plant material selected was not completely rotted, and the tissue removed included both healthy and diseased zones. Tissue platings were normally made on corn meal and water agar; pure cultures of *Pythium* spp were easily obtained when this procedure was followed

Pythium anandrum, P. oligandrum, and *P ultimum* were isolated first Further isolations yielded, also, *P irregulare* Buis and *P splendens* Braun for both of which rhubarb is a new host A detailed description and discussion of the above-mentioned species, together with their host range and geographic distribution, has already been given by Middleton (1943), so that only a brief presentation of specific information will be given here

Morphology. All five species named possess spherical sporangia Those of *Pythium anandrum* are typically borne terminally on rather long sporangiophores, and are prolate-ellipsoidal with an apical papilla; they thus bear some likeness to sporangia of species of *Phytophthora*, and measure about 32–85 µ (mean 50 µ) in length, and 18–40 µ (mean 25 µ) in width The sporangia of *P irregulare* are either acrogenous or intercalary, and are of various shapes, but mostly subspherical to obovate, though they may be ellipsoidal, pyriform, or truncate, usually measuring 10–27 µ (mean 18 µ) in diameter Sporangia of *P oligandrum* are of the contiguous type, formed of several more or less spherical units joined by connecting hyphal strands or merely sessile, one upon the other, the elements averaging 35 µ in diameter Relatively large acrogenous sporangia, spherical to subspherical, measuring 21–49µ (mean 36 µ) in diameter, with dense contents, typify *P splendens*. Acrogenous sporangia, borne on short lateral hyphae, typically spherical to subspherical, and measuring 12–28 µ (mean 20 µ) in diameter, are found in *P. ultimum*.

The oögonia of *Pythium anandrum, P irregulare*, and *P oligandrum* are spiny; those of *P. splendens* and *P. ultimum* are smooth The oögonia of *P anandrum* and *P. oligandrum* are very similar in size, ranging from approximately 15 to 35 µ (mean 27 µ) in diameter, and possess similar spines, which are conical in form and acutely tipped. Oögonia of *P. irregulare* may have numerous to few spines, and infrequntly lack spines, but in such instances the oögonial wall is undulate to somewhat blunt-serrate. Spines of *P. irregulare* vary in shape but are usually of broad base and acuminate apex, straight or contorted. *P. splendens* rarely produces oögonia; when present,

the oögonia are spherical and acrogenous; they vary in size from about 25 to 35 μ (mean 32 μ) in diameter, and possess a smooth, thin oogonial wall. Oögonia of *P. ultimum* are like those of *P splendens*, but smaller in size, ranging from 19 to 23 μ (mean 20 μ) in diameter.

Antheridia are lacking in *Pythium anandrum* and are rarely present in *P oligandrum*, then usually diclinous rather than monoclinous in origin, clavate and slightly crooknecked, with two or three transverse constrictions Antheridia of *P. irregulare* are typically monoclinous, and vary in number from 1–4 per oogonium. When the sexual apparatus of *P splendens* is available for study, usually 1–8 clavate and more or less crooknecked antheridia of mono- or diclinous origin are found Antheridia of *P. ultimum* are typically monoclinous, short, and sharply upcurved, originating immediately below the oogonium and making apical contact with the basal portion of the oögonial wall, the antheridial cell is not necessarily delimited by a septum

The oospores of *Pythium anandrum, P irregulare, P oligandrum, P splendens,* and *P ultimum* are aplerotic, with certain differences in diameters and in thickness of oospore walls

Pythium anandrum may be separated from its congeners by its sporangia and spiny oogonia *P oligandrum* is identifiable by its contiguous sporangia and spiny oogonia *P irregulare* is readily recognized by the peculiar echinulation of the oogonial wall *P splendens* is one of the few species of the genus that may be identified on the basis of its sporangial characters, though positive identification should rest upon observation of the sexual stage or certain physiologic traits *P ultimum* is generally determined on the basis of its spherical sporangia, thick-walled oöspore, and the more or less unique relationship existing between the oogonium and antheridium

Temperature—Growth Relations. The cardinal temperatures for growth of the several species of *Pythium* isolated from rhubarb were determined to be as follows (in degrees centigrade). *P anandrum:* minimum 1°, optimum 28°, maximum 34 , *P irregulare:* minimum 4°, optimum 28°, maximum 37°; *P oligandrum:* minimum 7°, optimum 31°, maximum 40°; *P splendens:* minimum 4 , optimum 28–31°, maximum 37°; *P ultimum:* minimum 1°, optimum 28°, maximum 37° These values conform with those given by Middleton (1943) for isolates of the species obtained from other hosts in various localities

PATHOGENICITY

The pathogenicity of the several *Pythium* spp to rhubarb seedlings and mature plants was determined Plants were grown from seed sown in flats containing sterile soil; seedlings were later transplanted to 6-inch pots of sterile soil In each test pure cultures of the fungus, grown on a mixture of sterilized whole oats and wheat, were added to the soil of three lots of 25

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THE PRODUCTION OF ANTIPHAGE AGENTS BY ACTINOMYCETES[1]

ALBERT SCHATZ AND DORIS JONES

The ability of microorganisms to inhibit bacteriophages is well known. With certain staphylococci, this antiphage effect may be due to the adsorption and destruction of the phage by cells of resistant strains derived from an originally susceptible culture (Rakieten 1932). The irreversible adsorption of staphylococcus bacteriophage has also been demonstrated with resistant strains of the *Bacillus subtilis* group (Rakieten, Rakieten & Doff 1936a). The capacity of bacteria other than staphylococci to adsorb or destroy staphylococcus phages has been proposed to explain, at least in part, why these phages are not commonly encountered in stool filtrates or sewage (Rakieten 1942). The phage-adsorbing capacity of yeasts and fungi has likewise been reported (Rakieten, Rakieten & Doff 1936)

In many investigations, substances which have been isolated from bacterial cells have been shown to possess phage-adsorbing or inactivating properties. Phospholipids obtained from susceptible and resistant strains of *Escherichia coli* as well as lipids of non-bacterial origin have been found inhibitive to bacteriophagia (Williams, Sandholzer & Berry 1940). A general rather than a selective inhibition has also been reported for various cholera phages with lipids from cholera strains. In these studies, the protein-free polysaccharide from susceptible smooth strains of cholera and El Tor vibrios selectively inhibited type phages lysing only the S form, while similar preparations from other serological groups or from R races did not prevent phage action (White 1936).

Extensive studies on bacterial cell products with phage-adsorbing properties have been carried out with shigellae (Burnet 1934), salmonellae (Levine & Frisch 1933), staphylococci (Burnet & Lush 1935), and streptococci (Tiffany & Rakieten 1939) among others. The objectives of these investigations were to study complex cell products (Levine & Frisch 1934), the immunological relationships of bacteria (Burnet & Lush 1935), or phage specificity (Levine & Frisch 1933). The antiphage agents were polysaccharides obtained from the susceptible homologous strains but generally not present in resistant cultures derived from secondary growth or in resistant

heterologous organisms. These substances inhibited bacteriophagia by adsorption with the virus, the effect usually being reduced or eliminated by previous exposure of the polysaccharides to antisera produced against the bacterial cells.

Effects of certain antibiotic agents on different bacterial viruses have also been studied. Penatin in the presence of glucose caused inactivation of an *E. coli* phage after an 18-hour exposure at room temperature. Although the inactivated particles retained their original morphology and were adsorbed by susceptible cells, they prevented colony formation by bacteria which the normal virus would ordinarily lyse (Anderson 1943). With streptomycin, streptothricin, clavacin, actinomycin, and penicillin it was found that a *S. aureus* phage and two *E. coli* phages were inactivated only by those agents inhibitive to gram-negative bacteria. Moreover, streptomycin-treated cells adsorbed phage whereas penicillin-treated bacteria did not (Jones 1945). Other investigations have shown that penicillin together with *S. aureus* phage K produced a more rapid killing than either agent alone; the multiplication and lytic action of the virus was not at all impaired by the antibiotic agent (Himmelweit 1945).

The object of the present investigations was to survey one group of microorganisms, namely the actinomycetes, for their ability to produce substances with antiphage properties and to isolate and study one such agent active against bacteriophages. The possibility of treating virus-infected bacteria with chemotherapeutic agents has been shown by the successful application of sodium arsenite to cells of *E. coli* after infection with phage γ. Arsenite concentrations of 7.5×10^{-3} M and over, when added to the phage-cell mixture during the latent period of intracellular virus growth, instantly inhibited virus multiplication, although they had no apparent effect upon bacterial reproduction (Spizizen 1943).

Whether microbial agents inhibitive to bacteriophagia will prove effective against animal and plant viruses remains to be determined. The fact that polysaccharides derived from *Aerobacter* sp. have been found to manifest phage-adsorptive ('Ashenburg, Sandholzer, Scherp & Berry 1940) as well as plant virus-inhibitive properties (Johnson 1941) may prove to be the exception rather than the rule.

MATERIALS AND METHODS

The actinomycetes studied in the present survey have been grouped for convenience as follows: (1) cultures chosen at random from different soils, manures, composts, pond water, and from the type culture collection; (2) organisms selected at random from a soil enriched with fowl-pox virus; (3) known antibiotic producers; and (4) actinomycetes isolated from various natural substrates by use of a selective phage–agar medium (Jones & Schatz

1946). In the last group, which consisted of 29 organisms, 17 were picked because the original colonies exhibited antiphage zones; the others were isolated because of antibacterial action rather than inhibition of bacteriophagia.

Each organism was grown for 8 days in stationary culture in (1) peptone–meat extract or nutrient broth, (2) glucose-tryptone, and (3) glycerol–yeast extract media containing 0.2 per cent agar. Certain of the organisms which failed to make satisfactory growth under static conditions were cultured for 5 or 7 days in shaken flasks. The filtrates, after heat treatment at 70° C for 10 minutes, were tested by the agar tube dilution technic (Jones & Schatz 1946) with an overnight exposure period at 37° C against *E. coli* LS2 phage and *S aureus* phage K. Any filtrates active against either or both of these phages were then tested against the *E. coli* PC phage. With the agar tube dilution procedure, the end point was that dilution of culture filtrate which produced at least a 100-fold reduction in plaque titre. Most of the active filtrates were further studied by the agar diffusion or cup method. Phage antagonism by the actinomycetes was determined by the streak test procedure.

EXPERIMENTAL RESULTS

A total of 176 strains of actinomycetes, comprising various members of the genera *Streptomyces*, *Nocardia*, and *Micromonospora*, as well as one culture of *Mycobacterium phlei*, were tested. The results indicate that 49 of these 176 actinomycetes, or 28 per cent, produced antiphage activity in filtrates from at least one of the three culture media on which each organism had been grown. Since the heat treatment used for sterilization of the culture broths may have destroyed some thermolabile active substances, it is possible that the actual number of actinomycetes which produce antiphage filtrates may exceed 28 per cent.

Table 1 presents more detailed information on the distribution of antiphage properties among the actinomycetes as determined by the agar tube dilution technic. Of 147 organisms tested on the three media, 39, or 26 per cent, were active Only 57, or 13 per cent, of a total of 441 filtrates inhibited bacteriophagia. While most active filtrates produced a 100-fold reduction of phage titre only in a dilution of 1:2, 8 filtrates were as active at a dilution of 1:20, 2 were as active at 1:100, and one filtrate was active even at 1:200. Of the 57 active preparations, 30, or 53 per cent, inhibited the staphylococcus phage K as against 14, or 25 per cent, which were active against the *E. coli* LS2 phage. Only one filtrate was active against all three phages, while 12 inhibited two of the three phages. This selectivity of antiphage action is comparable to the selective antibacterial properties of antibiotic agents. Since only those filtrates which affected the action of the LS2 and K phages were tested against the *E. coli* PC phage, it is likely that a greater number of filtrates would have been found active if all had been tried against the PC phage.

In addition to their selective action, antibiotic and antiphage substances are similar in that the production of each is dependent to a large degree upon the composition of the medium. With antibiotics, this is well known, the specific substances required for the formation of highly active antibacterial filtrates having been designated as "activity factors" (Waksman, Schatz & Reilly 1946). Of the 39 actinomycetes reported in table 1, two were active on all three media, while 14 produced antiphage filtrates on both the tryptone-glucose and glycerol-yeast extract media. No organism was active only on the peptone–meat-extract substrate, but 9 actinomycetes were active only on the glucose and 14 only on the glycerol medium.

TABLE 1. *Antiphage properties of active culture filtrates of thirty nine actinomycetes.*[a] Agar tube dilution technic.

Medium	End point, active filtrate dilution[b]	Number of filtrates active against only									Total active filtrates
		S3K	LS2	LS2, PC, and S3K	LS2 and PC — LS2	LS2 and PC — PC	PC and S3K — PC	PC and S3K — S3K	LS2 and S3K — LS2	LS2 and S3K — S3K	
Peptone-meat extract	1 : 2	2									2
Glucose-tryptone	1 : 2	15	8				2		3	2	
	1 : 20	2						2		1	30
Glycerol yeast extract	1 : 2	8	6	1	2	2	2	1	1		
	1 : 20	2						1			
	1 : 100								2	2	
	1 : 200	1								1	25
Total		30	14	1	2		4		6		57

[a] Of 147 organisms tested in three different media, 39 were active.
[b] Dilution giving a 100 fold reduction in plaque titre after overnight exposure at 37° C.

Although most unheated active filtrates retained their potency when refrigerated at 4° C for three weeks, some preparations decreased in activity while others became completely inactive. Moreover, some originally active actinomycetes appeared to undergo a progressive loss of ability to produce antiphage filtrates. Studies with antibiotic agents have demonstrated similar phenomena, namely, loss of antibacterial activity in microbial filtrates and deterioration in potency of a parent culture.

That inhibition of bacteriophagia was not a pH effect was evidenced by the fact that few culture filtrates, if any, were significantly acid and that these were brought well above the critical range when mixed with the nutrient broth phage suspension. In view of the reported adsorption of phage by yeasts and molds (Rakieten, Rakieten & Doff 1936b) and the small number of active filtrates which inhibited bacteriophagia by the cup test, there is a

possibility that the phage inhibition might have been due to adsorption on spores and small mycelium fragments, some of which undoubtedly passed through the paper filters. However, the minute amount of such particles present in the clear culture filtrates as well as the dependence of antiphage action on composition of the medium would appear to eliminate this likelihood.

When tested by the agar diffusion or cup method, no active filtrates produced an appreciable antiphage zone against either of the *E. coli* phages. Of 39 filtrates which acted on the staphylococcus phage by the agar tube dilution technic, 7 produced large zones in which bacteriophagia was affected. The diameters of these zones were 33, 24, 26, 21, 17, and 35 mm. Only one of the zones contained an inner ring of antibacterial action surrounding the cup.

TABLE 2. *Antiphage and antibacterial activity of active actinomycetes.*
Agar streak technic.[a]

Agar seeded with phage	Number of tests	Inhibition zones[b]			
		Antibacterial only	Antiphage only	Both anti bacterial and antiphage	Absent
E. coli PC	7	2	1	3	1
E. coli LS2	11	3	6		2
Staph. aureus K	35	12	6	5	12
Total	53	17	13	8	15

[a] Cultures which produced active filtrates in liquid media were streak tested against the susceptible phages on the corresponding 1% agar media.
[b] Zones ranged from 1 to 23 mm.; antiphage zones less sharply defined and therefore less accurately measured than antibacterial zones.

All zones showed more or less complete phage inhibition except the last, in which the size rather than the number of plaques was reduced.

For the streak test, actinomycetes which produced activity in broth were tested on the corresponding agar media against those phages sensitive to the culture filtrates. The results given in table 2 reveal that in 53 such tests 17 plates showed only antibacterial zones, 13 exhibited only antiphage zones, and 8 had antibacterial areas within antiphage zones; 15 plates revealed neither type of activity. Since antibacterial action was obtained, with or without antiphage action, in 25 of the 53 tests, one might assume that in many cases the effect of the culture filtrates on bacteriophagia was due to an effect on the cells rather than on the phages by antibiotic agents present in concentrations sub-inhibitive for bacterial growth.

Of the known antibiotic-producing actinomycetes tested on the three media, *N. gardneri*, *S. antibioticus*, and *S. lavendulae* failed to exhibit antiphage properties, although certain filtrates were antibacterial. On the other hand, some streptomycin-producing strains of *S. griseus* as well as an asporo-

genous inactive variant (Schatz & Waksman 1945) were found to yield cul-
ture filtrates highly active against phage. Other sporogenous strains, No. 42.1
and 3326a, did not exhibit antiphage action, although the former produced
streptomycin while the latter did not (table 3). Moreover, a strain such as
No. 19 produced antibacterial but not antiphage activity in certain media
while the reverse was true with other substrates. These results together with
the observation that different preparations of streptomycin varied in their
activity upon *E. coli* PC phage (Jones 1945) indicated that the antiphage
effect of certain streptomycin lots might be due to some substance other than
the antibacterial agent itself.

TABLE 3. *Activity of different strains of S. griseus against bacteria and S. aureus phage K.*

Agar tube dilution test. 15-day culture filtrates.

S. griseus strain	Medium[a]	*E. coli* units per ml., cup test	Phage survival at filtrate dilution[b]			
			1 : 2	1 : 20	1 : 100	1 : 200
19 (sporogenous)	DA	0	++++	++++	+++	++++
	TG	14	AB	+++	++++	+++
	YG	0	+	+	++	++
	GN	16	AB	+++	++++	++++
42.1 (sporogenous)	DA	0	++++	++++	++++	++++
	TG	23	AB	++++	++++	++++
	YG	40	AB	++	++++	+++
	GN	45	AB	++	++++	++++
Variant 3 (asporo- genous)	DA	0	++++	++++	++++	+++
	TG	0	+	++	++	++
	YG	0	+	++	++	++
	GN	0	+	+	+	+
3326a (sporogenous)	TG	0	++++	++++	++++	++++
	YG	0	++++	++++	++++	++++
	GN	0	++++	++++	++++	++++

[a] DA = dextrose asparagin; TG = tryptone glucose; YG = yeast extract glycerol; GN = glucose-nutrient broth. All media contained 0.2% agar.
[b] AB = antibacterial action; ++++ = normal plaque titre; ++ = a 10 fold reduction in plaque titre; + = a 100-fold reduction or more in plaque titre.

Comparisons were, therefore, made of the antiphage and antibacterial
properties of several lots of streptomycin which differed considerably in
potency. These were dissolved in nutrient broth and neutralized, and the
resulting solutions were sterilized by heating at 75° C for 10 minutes before
being tested against *S. aureus* phage K. Approximately 1,500 µg. of strepto-
mycin contained in 1.5 ml. were mixed with an equal volume of phage sus-
pension of such titre that plaque counts of surviving phage were made at
dilutions where antibacterial activity was eliminated. As shown in table 4,
the hydrochloride lots were characterized by considerably greater antiphage
action than the formate samples, although the antibacterial activity per ml.,
as determined by cup assay, was about the same throughout.

The difference between the two streptomycin salts suggested that the formate ion per se might affect antiphage action or that the formate preparations might contain some other factor which inhibited the phage-inactivating agent. The former possibility was eliminated by the observation that a solution of streptomycin hydrochloride (300 µg./ml.) to which 1.7 mg. sodium formate per ml. had been added retained its antiphage activity when tested after incubation overnight at 37° C.

On the other hand, a formate salt of streptomycin was found definitely to interfere with the phage-inactivating ability of one of the hydrochloride preparations. For this experiment, phage was exposed to the hydrochloride, formate, and a mixture of the two in which 50 per cent of the antibacterial activity was contributed by each preparation. The results revealed that 510

TABLE 4. *Activity of different streptomycin preparations against S. aureus phage K.*

Streptomycin preparation	E. coli units per mg.[a]	Phage exposed to units per ml[a]	Surviving phage, particles per ml.
Formate	19	520	27×10^5
Formate	45	500	14×10^5
Forma.e	58	500	50×10^4
Formate	110	480	10×10^5
Hydrochloride[b]	100	510	21×10^2
Hydrochloride[b]	400	450	35×10^2
Nutrient broth control	0	0	15×10^6

[a] Cup assay.
[b] Obtained through the courtesy of Merck and Co., Inc., Rahway, N. J.

µg. per ml. of the hydrochloride alone caused approximately a 10,000-fold reduction in phage titre. On the other hand, when phage was exposed to 1000 µg. per ml. of the formate-hydrochloride mixture, in which both preparations were equally present on the basis of antibacterial unitage, the titre was decreased only about 10-fold. This same order of activity was found with 500 µg. per ml. of the formate alone.

Since the hydrochloride lots were obtained from Merck & Co., Inc., while the formate samples were prepared in our own laboratory, it is difficult to interpret the data because the two types of products very probably differed in respect to strain of *S. griseus*, composition of medium, conditions of growth, and method of recovery of streptomycin. However, the fact that an antiphage agent is obtained with strains which do not produce streptomycin as well as with streptomycin-producing strains in media where no antibacterial substance is formed (table 3) indicates that the antiphage action is due to a product of *S. griseus*. This substance cannot be isolated by the charcoal adsorption acid-alcohol elution technic used to recover streptomycin. It differs from the second antibiotic agent isolated from *S. griseus* (Waksman,

Schatz & Reilly 1946), since an active preparation of this lipid-like substance has been found to lack activity against bacteriophage.

For detailed study, *Streptomyces* No. 193 was chosen because it produced filtrates highly active against the *E. coli* PC and *S. aureus* bacteriophages, although the same filtrates possessed no antibacterial properties when tested by the agar dilution method against *E. coli*, *S. aureus*, *B. subtilis*, and *B. mycoides*. The parent culture, which had been isolated from a fowl-pox-enriched soil, underwent marked variation. The original organism upon serial transfer repeatedly grew in distinct areas of white and mouse-gray on dextrose-asparagin agar slants. By plating out and single colony selection, a pure white and a mouse-gray, or brown, strain were obtained. Both of these

TABLE 5. *Antiphage activity of culture filtrates of Streptomyces sp. No. 193 in different liquid media.*

Agar tube dilution test

| Medium[a] | Phage survival at filtrate dilutions[b] | | | | | |
| | *S. aureus* phage K | | | *E. coli* PC phage | | |
	1 : 2	1 : 20	1 : 200	1 : 2	1 : 20	1 : 200
Peptone meat extract	+	+	++++	+	+++	++++
Peptone-meat extract glucose	+	+	++++			
Peptone-glucose		+	++++			
Tryptone starch	0	0	++	0	+	+++
Yeast extract-glycerol		+++	++++			
Sodium nitrate glucose corn steep liquor		+	+++			
Glutamic acid glucose		+	+			
Yeast extract-glucose casein		++++	++++			

[a] Mineral salts plus 0.2% agar in each medium.
[b] 9 day-old culture filtrates tested. ++++ = no plaque reduction; ++ = a 10 fold reduction in plaque titre; + = a 100-fold reduction in plaque titre; 0 = no viable phage, complete inactivation.

were stable and active with about the same antiphage spectra; both produced culture filtrates devoid of antibacterial action. In addition to these strains, a sporulating variant with a purple reverse, a black sporogenous variant, and an asporogenous, nocardial form were obtained. Of these three types, which were stable upon transfer, the first was found to be inactive against bacteriophages and bacteria; the last two were not tested. Variations similar to these have been observed with the actinomycetes that produce streptothricin and streptomycin (Schatz & Waksman 1945; Waksman & Schatz 1945).

In determining the activity of No. 193 in different media, antiphage action was obtained with several substrates, as illustrated in table 5. However, only the glucose-tryptone and glucose-glutamic acid media were used. In stationary cultures, the actinomyces produced filtrates which by the agar tube dilution method gave a 100-fold reduction of phage titre at dilutions of 1 : 20 to 1 : 100, the end-point varying with different lots. The active agent

could be concentrated from the culture filtrate by adsorption on charcoal and subsequent elution with a 1:1 mixture of ether and 95 per cent ethyl alcohol, although the percentage of recovery was rather low. The antiphage substance does not decrease in activity if heated for 10 minutes at 70–80° C. However, Seitz filtration causes some loss in potency. Considerably greater activity was obtained by extraction of moist 9-day-old pellicles with ether-alcohol. Upon removal of the organic solvents, the residue formed a stable aqueous suspension. Some such preparations by the agar tube test produced 100-fold reductions in plaque titre of *S. aureus* phage K at dilutions of about 1:200,000. On a dry weight basis this represented dilutions of approximately 1:1,000,000.

It appears that the antiphage agent acted directly upon the phage particles rather than through an effect on the host cells, since it was found that a pre-exposure of phage to agent was required and that the degree of inactivation for a given phage titre varied within limits with the concentration of the agent. The fact that untreated phage produced normal lysis when plaque-counted with semisolid agar to which the agent had been added immediately before plating indicates that once a phage particle is adsorbed on or has entered into a host cell, the antiphage agent can not inhibit its multiplication and subsequent lytic effect.

When tested against fowl pox virus, the 193 concentrate was active in vitro but not in vivo. With 11-day-old chick embryos, it was found that the agent, undiluted as well as diluted 1:20, completely inactivated the virus after overnight exposure at room temperature. However, no protective effect was obtained when infected eggs were treated with the agent or when virus and agent were injected simultaneously but separately.

SUMMARY AND CONCLUSIONS

The antagonistic action of certain microorganisms upon bacteriophages and the inhibitive effects of substances of microbial origin upon bacteriophagia are well known. The present study concerns phage antagonism by actinomycetes and the production of an antiphage agent by a representative of this group of microorganisms.

Of 176 actinomycetes isolated by different means from a variety of substrates and grown in three media, 28 per cent gave culture filtrates active against one or more of three phages when tested by the agar tube dilution technique. Antiphage activity depended upon composition of the culture medium and was selective in nature. Both of these aspects are well known with antibiotic agents. Of the cultures which produced antiphage filtrates by the agar tube test, not all were active by the streak test. Some were only antibacterial; others were only antiphage. Certain organisms exhibited an antibacterial within an antiphage zone; several actinomycetes lacked either

type of action. Of the culture filtrates active by the agar tube dilution method, several produced zones of phage inhibition only against the staphylococcus bacteriophage when cup-tested.

Of the known antibiotic-producing actinomycetes, only *S. griseus* produced antiphage filtrates. The active agent was not streptomycin, since it was obtained with strains which did not produce this antibiotic as well as with streptomycin-producing strains cultured in certain media where no antibacterial substance was formed. Therefore, the nocardia-like variant and certain related streptomyces strains which have been found to differ on the basis of their capacity to produce streptomycin cannot be separated by their ability to produce the antiphage agent.

While two samples of streptomycin-hydrochloride were active against phage, four formate preparations were not. Moreover, the impure streptomycin-formate contained some substance which antagonized the antiphage action of the hydrochloride.

One active species of *Streptomyces*, isolated from a soil enriched with fowl-pox virus, was studied in detail. This organism produced in several media a thermostable antiphage agent active against *S. aureus* phage K and *E. coli* PC phage, but not against *E. coli* LS2 phage. The substance could be concentrated by adsorption on charcoal and subsequent elution with ether-alcohol, although considerably greater activity was obtained by ether-alcohol extraction of the mycelium of the organism. The agent formed a stable aqueous suspension which inhibited the staphylococcus bacteriophage at a dilution of about 1 : 1,000,000 on a dry weight basis. From the experimental results, it is believed that the effect of the antiphage agent is on the phage particles themselves rather than on the host cells, and that once infection has taken place the agent does not interfere with phage multiplication.

The 193 concentrate inactivated fowl-pox virus in vitro but was ineffective in vivo.

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THE ROLE OF CERTAIN ENVIRONMENTAL FACTORS IN GROWTH AND REPRODUCTION OF PROTOSIPHON BOTRYOIDES KLEBS—II. VEGETATIVE GROWTH[1]

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INFLUENCE OF ILLUMINATION

Two series of experiments were set up to determine the effect on growth of *Protosiphon* of different lighting periods. Six flasks of 0.06 per cent Bristol's solution and six tube slants with the same solution in 1.5 per cent agar and inoculated with clone *8B2* were used for each experiment in one series and the same medium with the addition of 0.25 per cent glucose was used for the second series. One set of six slants and six flasks with inorganic medium and a similar set containing glucose were kept in total darkness for three months. Additional sets with and without glucose were kept in light-tight boxes except during daily lighting periods of four, eight, ten, twelve, and twenty-four hours.

The longest sacs were produced on slants that were exposed to a ten-hour period of daily illumination. In every case the mean length of one hundred sacs and the amount of centrifugate per flask was greater in the glucose-containing medium. In all cases too the total amount of centrifugate increased with the length of the period of daily illumination. From flasks with inorganic liquid medium, three times as much material was centrifuged from flasks grown under twenty-four hours daily illumination as from flasks with a four-hour lighting period. In the corresponding series containing 0.25 per cent glucose, the amount of centrifugate was four times as great from flasks lighted twenty-four hours as from those with four hours of light daily.

There was a thin layer of growth, green in color and macroscopically visible, on slants with inorganic medium that were kept in total darkness for three months. Microscopic examination showed that the growth consisted of small spherical cells and numerous zygotes. The results indicate that very small amounts of nutrient material are sufficient to sustain growth of *Protosiphon* for three months. These were probably introduced with the inoculum or available as impurities in the agar (Robbins 1939). On the slants with 0.25 per cent glucose added to the inorganic medium, there was a thick layer of red coenocysts. In the flasks containing glucose medium, there was a thick layer of red coenocysts also macroscopically visible. The experiment indicates that considerable growth may occur in darkness when carbohydrate is sup-

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[1] Part I of this series appeared in Bull. Torrey Club 73: 000–000. Numbering of illustrations is continuous throughout the series. A list of literature cited will appear in part III.

plied. It also shows that light is not requisite for formation of red coenocysts, and, inasmuch as zygotes were present in all the cultures, that conjugation takes place readily in darkness.

INFLUENCE OF THE CULTURE MEDIUM—PHYSICAL FACTORS

A. Salt Concentration of the Medium. Klebs (1896), Livingston (1900, 1901, 1905), Artari (1904, 1906), Pringsheim (1914, 1926a) and others have demonstrated that the concentration of the solution in which *Protosiphon* and other organisms are cultured has a decided effect on their growth and reproduction. In order to obtain information on the optimum concentration of culture media for the growth of clonal cultures, growth was observed in a number of different culture media of varying concentrations. The first five media used in table 6 are listed by Bold (1942). Slight changes, such as the addition of minor elements, were made, and the formulae are listed here as they were used in the experiments.

Beyerinck's Solution, 1898

Distilled H₂O	100.0 ml.	1% FeCl₃ solution 2 drops per liter
NH₄NO₃	0.05 gram	minor elements solution 2 ml. per liter
K₂HPO₄	0.02 gram	molybdic acid solution 1 ml. per liter
MgSO₄·7H₂O	0.02 gram	
CaCl₂·2H₂O	0.01 gram	

= 0.1% solution by weight

Bristol's Solution, 1919

KH₂PO₄	0.7 gram	Distilled H₂O	1000.0 cc.
K₂HPO₄	0.3 gram	1% FeCl₃ solution	4 drops
NaNO₃	1.0 gram	minor elements solution	2 ml.
CaCl₂	0.1 gram	molybdic acid solution	1 ml.
MgSO₄	0.3 gram		
NaCl	0.1 gram		

= 0.25% solution by weight

Detmer's Solution, 1888

Ca(NO₃)₂	1.0 gram	Distilled H₂O	1000.0 ml.
KCl	0.25 gram	1% FeCl₃ solution	3 drops
MgSO₄	0.25 gram	minor elements solution	1.5 ml.
KH₂PO₄	0.25 gram	molybdic acid solution	0.75 ml.

= 0.175% solution by weight

Knop's Solution, 1865 (from Bold, 1942)

Part A		Part B	
Ca(NO₃)₂	4.0 gram	KNO₃	1.0 gram
Distilled H₂O	500.0 ml.	KH₂PO₄	1.0 gram
1% FeCl₃ solution	3 drops	MgSO₄·7H₂O	1.0 gram
minor elements solution	2 ml.	Distilled H₂O	500.0 ml.
molybdic acid solution	1 ml.		

= 0.7% solution by weight

Lefévre's Solution, 1937

KNO_3	0.2 gram	Distilled H_2O	1000.0 ml.
K_2HPO_4	0.4 gram	1% $FeCl_3$ solution	2 drops
$MgSO_4$	0.25 gram	minor elements solution	1 ml.
$Ca(NO_3)_2$	0.25 gram	molybdic acid solution	0.5 ml.

= 0.11% solution by weight

Pringsheim's Solution, 1926a

KNO_3	0.1 %	The following were added to each	
$MgSO_4 \cdot 7H_2O$	0.01%	liter of solution:	
$(NH_4)_2HPO_4$	0.02%	1% $FeCl_3$ solution	2 drops
$CaSO_4$	0.01%	minor elements solution	1 ml.
		molybdic acid solution	0.5 ml.

= 0.14% solution by weight

Minor elements stock solution
Craig and Trelease, 1937

$ZnSO_4 \cdot 7H_2O$	100 mgm.	$CuSO_4 \cdot 5H_2O$	3 mgm.
H_3BO_3	100 mgm.	Distilled H_2O	1000.0 ml.
$MnSO_4 \cdot 4H_2O$	150 mgm.		

Molybdic acid solution

85% molybdic acid	3 mgm.
Distilled H_2O	1000.0 ml.

Four concentrations of each solution were inoculated with clone 8B2. Six flasks each containing 35 ml. of solution were used for each concentration and the cultures were permitted to grow for fifty days. The results are summarized in table 6.

In the experiments with liquid media, the amount of centrifugate per flask is a better criterion of the amount of growth than the length of sacs. Some media tend to stimulate repeated gamete formation and in such cases no long sacs develop. In table 6, the longest sacs were found in 0.1 per cent Beyerinck's solution, but the total amount of material centrifuged from flasks with this medium was less than the total from any other medium except Pringsheim's.

The largest amount of material was centrifuged from the flasks with Bristol's solution. Detmer's solution gave the next largest yield and the sacs were a little larger than those in Bristol's solution. The pH of Bristol's solution changed less than that of any of the solutions used and this is probably the most important advantage in its use. The pH of Detmer's solution at the beginning of the experiment was lower than that of a freshly prepared solution because it had been standing. There was no significant difference in size of the sacs in any of the concentrations of Detmer's solution, but the highest concentration used was 0.175 per cent. The higher concentrations of Bristol's solution (0.25%) and Knop's solutions (0.7%) increase the possibility of

osmotic effects (Livingston 1900). The final hydrogen-ion concentration of the more dilute concentrations of Beyerinck's solution was high and the red color of the coenocysts present was visible to the naked eye.

TABLE 6. *Influence of the concentration of the medium on growth at an average temperature of 16.5° C of clone 8B2.*
(In all experiments except where otherwise noted the pH was determined by means of a glass electrode.)

Solution	Per cent conc. by weight of solution	pH		Color intensity	Amount centrifuged from one flask (ml.)	Mean length of 100 cells after 50 days (mm.)
		At beginning of experiment	After 50 days			
Beyerinck's 1898	0.10	7.0	5.3	2	0.05	0.52
	0.05	6.8	3.8	1	0.02	0.13
	0.025	6.5	3.7	1	0.03	0.05
	0.0125	6.2	3.7	0	0.01	0.04
Bristol's 1919	0.25	6.2	6.2	2 +	0.07	0.13
	0.125	6.3	6.4	2	0.06	0.12
	0.06	6.3	6.5	2	0.06	0.20
	0.03	6.3	6.6	2	0.04	0.20
Detmer' 1888	0.175	4.5	5.7	2	0.05	0.23
	0.08	4.2	6.3	2	0.06	0.20
	0.04	4.0	6.3	2	0.05	0.25
	0.02	4.0	6.8	2	0.06	0.25
Knop's 1865	0.70	3.9	4.0	0	0.01	0.03
	0.35	4.3	5.1	1	0.04	0.12
	0.175	4.6	5.8	2	0.05	0.12
	0.09	4.4	6.0	2	0.07	0.15
Lefévre's 1937	0.11	7.4	7.0	2	0.05	0.07
	0.055	7.4	7.2	2	0.05	0.17
	0.03	7.2	7.8	1 +	0.05	0.06
	0.015	6.9	7.2	1	0.03	0.08
Pringsheim's 1926a	0.14	6.8	6.2	1 −	0.01	0.03
	0.07	6.8	4.4	1	0.04	0.12
	0.035	6.8	5.5	1	0.03	0.12
	0.018	6.7	4.8	1 −	0.01	0.06

B. Concentration of Agar. Moewus (1933) reports that concentrations of 0.7–1.0 per cent agar evoke the production of large sacs in *Protosiphon*, while higher concentrations are inhibiting in this respect. Pringsheim's and Bristol's solutions were selected for testing the effect of agar concentration on the growth of *Protosiphon* because good results had been secured with them in other experiments. Table 7 and figure 18 list the agar concentrations used and the mean length of sacs for the different observation periods. At the end of twelve days, the longest sacs in both solutions were produced on agar in 0.75 per cent concentration, while the size decreased markedly in lower and higher concentrations.

At the end of twenty-four days, the longest sacs occurred in 0.5 per cent agar in Bristol's solution and on 0.75 per cent agar in Pringsheim's solution.

Sacs from 0.06 per cent Bristol's solution without agar were mostly spherical (fig. 22). The mean lengths of sacs from either 0.5 per cent or 0.75 per cent agar (fig. 23) were approximately six times as great as the mean length of sacs from 4.5 per cent agar concentration (fig. 24). Sacs from 2.5 per cent agar were more than three and one-half times as long as those from 4.5 per cent agar concentration.

The mean lengths of sacs were not as great when Pringsheim's solution was used, but the form of the curve in figure 18 indicates the effects of agar concentration were essentially the same with the two media. On Pringsheim's solution, the longest sacs at the ends of both the twelve- and the twenty-four-

TABLE 7. *Effect of agar concentration on cell size of clone 8B2 cultures at an average temperature of 17° C in 0.06 per cent Bristol's and 0.06 per cent Pringsheim's solutions.*

Agar conc. (per cent)	Mean length of 100 sacs in mm. after					
	12 days	24 days	40 days	12 days	24 days	40 days
	0.06 per cent Bristol's solution			0.06 per cent Pringsheim's solution		
0.0	0.04	0.09	0.14	0.05	0.06	0.20
0.25	0.05	0.20		0.10	0.15	0.60
0.5	0.55	**0.94**	0.5	0.19	0.25	0.75
0.75	**0.6**	0.88		**0.30**	**0.75**	1.00
1.0	0.45	0.77	0.87	0.20	0.55	1.00
1.5	0.27	0.75	**1.30**	0.15	0.40	1.00
2.0	0.25	0.75	0.87	0.17	0 40	0.88
2.5	0.20	0.55	0.75	0.17	0.25	0 65
3.0	0.10	0.44	0.75	0.15	0.21	0.37
3.5	0.03	0.19	0.75	0.15	0.20	0.25
4.0	0.05	0.12	0.75	0.06	0.12	0.23
4.5	0.03	0.15	0.60	0.03	0.12	0.20
5.0	0.03	0.12	0.45	0.04	0.12	0.21

day periods were on the slants with 0.75 per cent agar. Sacs with two or more lobes separated by septa were common in cultures with less than 1 per cent agar. In 0.06 per cent Pringsheim's solution in liquid form, most cells were spherical just as they were in Bristol's solution in liquid form. After twenty-four days growth on agar concentrations higher than 0.75 per cent, the mean lengths of the sacs were less than on 0.75 per cent, but the diameter of the aerial portions was greater (figs. 25–26).

These observations for a growth period of twenty-four days support Moewus' observation (1933) that lower concentrations of agar effect increased cell size, apparently by stimulating increased growth of the rhizoidal portion of the cell. This is probably related to thigmotropic or geotropic stimuli. Higher concentrations of agar retard growth initially, but this effect is overcome to some extent in older cultures. For general use however, cultures inoculated on agar concentrations of 0.5 or 0.75 per cent are difficult to handle because the agar breaks so easily. With 1 or 2 per cent agar, the dishes can be moved around without undue disturbance of the culture.

After forty days, the plants in Bristol's solution in agar concentrations of 3–5 per cent were still in vegetative condition and growing. The largest sacs were on slants solidified with 1.5 per cent agar. On agar concentrations below 2 per cent, the contents of most of the sacs had formed coenocysts. No figure is recorded in table 7 for the mean length of sacs from cultures with 0.25 per cent agar or 0.75 per cent agar added to Bristol's solution and allowed to grow for forty days, because only small coenocysts were observed in these cultures. The larger sacs in these cultures evidently were stimulated to swarmer formation after the twenty-four-day observations were made.

On Pringsheim's solution, sacs were equally long after 40 days on 0.75 per cent, 1.0 per cent, and 1.5 per cent agar. On concentrations of agar of 3.5–5 per cent there was a significant increase in length of sacs between the 24- and 40-day growth periods. Most of the sacs in all the cultures in Pringsheim's solution were in vegetative condition and therefore still growing.

INFLUENCE OF THE CULTURE MEDIUM—CHEMICAL FACTORS

The role of various chemical elements in the nutrition of algae has been extensively investigated by such workers as Artari (1904, 1906, 1913), Chodat (1913), Pringsheim (1912, 1914, 1926a), Luksch (1932), and others.

The present report deals only with such factors as the effect of various culture media, the effect of minor elements, including molybdenum, of different nitrogen sources, and of hydrogen-ion concentration on the growth and reproduction of *Protosiphon*.

A. Inorganic media

1. Experiments with Various Media. Beyerinck's, Bristol's, Detmer's, Lefévre's, and Knop's solutions (p. 21, 22) were prepared in concentrations of 0.06 per cent by weight. The hydrogen-ion concentration of Knop's and of Detmer's solutions was adjusted to pH 6.0 by adding N KOH. The solutions were then resterilized. From each medium, six slants solidified with 1.5 per cent agar and six flasks each containing 35 ml. of liquid medium were inoculated with clone 2*B*1 and grown for 24 days at an average temperature of 20° C.

The temperature was too high to permit growth of long sacs; the mean length of sacs on slants made from Beyerinck's solution was only 0.04 mm., and on the other media approximately 0.20 mm. Five-hundredths ml. of centrifugate were obtained from a flask of Beyerinck's solution and about 0.03 ml. from a flask with each of the other solutions. Although rather crude, these data indicate that good growth of *Protosiphon* may be secured in a number of media commonly used for the cultivation of algae. The final hydrogen-ion concentration of Beyerinck's solution was high (see table 6).
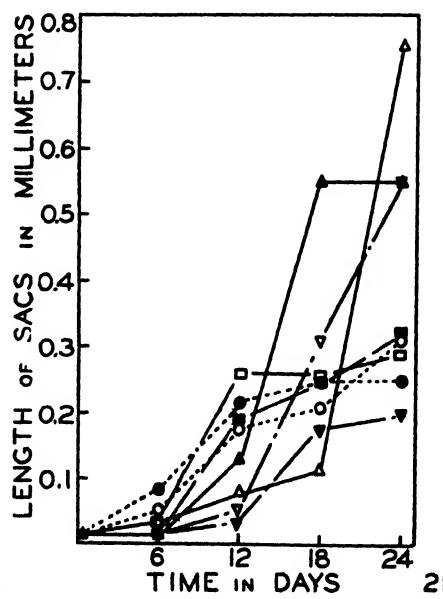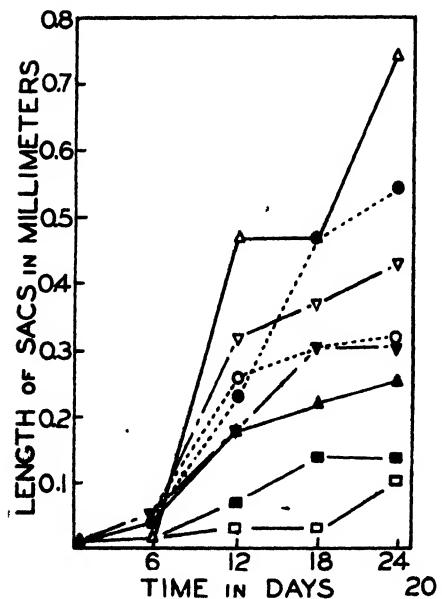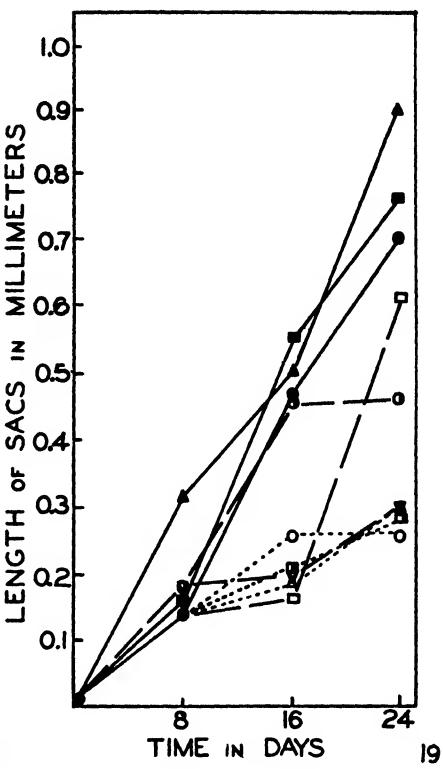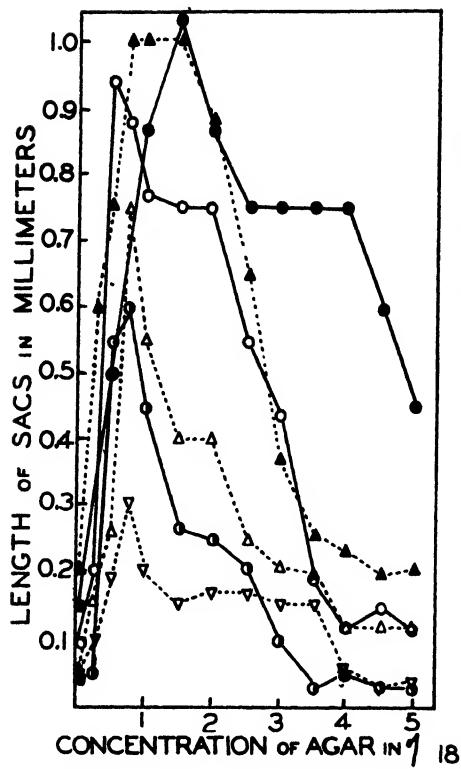
This characteristic makes the solution less desirable for use. The same objection prevails for Detmer's and Knop's solutions if they are made according to the original formulae.

2. Experiments on the Form of Nitrogen Required.

The increase in hydrogen-ion concentration of Beyerinck's solution indicates that ammonium nitrogen is being utilized. In an attempt to determine the effect of different nitrogen sources on the growth of *Protosiphon* and on the hydrogen-ion concentration of the solutions, Detmer's, Bristol's, and Pringsheim's solutions were prepared with NO_3/NH_4 ionic ratios of 100/0, 50/50, and 0/100. The total nitrogen concentrations and the weight of salts used was kept approximately unchanged for each solution. The different NO_3/NH_4 ratios were obtained by varying the proportions of salts containing nitrogen as follows.

Explanation of figures 18–21

FIG 18. Graph showing the effect on length of sacs in clone 8B2 of different concentrations of agar used to solidify 0.06% concentrations of Bristol's and of Pringsheim's solutions.

————•———— length of sacs after 12 days in 0.06% Bristol's solution.
————◦———— length of sacs after 24 days in 0.06% Bristol's solution.
————•———— length of sacs after 40 days in 0.06% Bristol's solution.
  • . length of sacs after 12 days in 0.06% Pringsheim's solution.
  . ▲ length of sacs after 24 days in 0.06% Pringsheim's solution.
  ▲ length of sacs after 40 days in 0.06% Pringsheim's solution.

FIG. 19. Effect of different NO_3/NH_4 ionic ratios with 0.06% concentrations of various media on the growth of clone 8B2 on slants with 1.5% agar at a temperature of 17.5° C.

	Solution	Ionic ratio		Solution	Ionic ratio
————•————	Bristol's	100/0	··· ◦ ···	Bristol's	0/100
————•————	Detmer's	100/0	· ▲·····	Detmer's	0/100
————•————	Pringsheim's	100/0	··· ◦ ···	Pringsheim's	0/100
— —•— —	Bristol's	50/50			
— —ı— —	Detmer's	50/50			
— —◦— —	Pringsheim's	50/50			

FIG. 20. Effect of 0.25% and 0.5% concentrations of various carbohydrates added to 0.06% Bristol's solution solidified with 1½% agar on the growth of clone 2B1 at a temperature of 20½° C.

· ··•· ···	0.25%	dextrose	— · —	0.25%	sucrose
· ◦ ··	0.5%	dextrose	— · —	0.5%	sucrose
————•————	0.25%	inulin	————•————	0.25%	xylose
————•————	0.5%	inulin	————◦————	0.5%	xylose

FIG. 21. Effect of addition of 0.25% and 0.5% concentrations of fructose, maltose, mannose and raffinose to 0.06% Bristol's solution with 1.5% agar on the length of sacs of clone 2B1 at a temperature of 20° C.

————•————	0.25%	fructose	— · —	0.25%	mannose
————•————	0.5%	fructose	— · —	0.5%	mannose
······•····	0.25%	maltose	————•————	0.25%	raffinose
······◦ ·	0.5%	maltose	————•————	0.5%	raffinose

Bristol's Solution

Four drops of 1% aqueous solution of $FeCl_3$, 2 ml. of minor elements solution (Craig and Trelease, 1937) and 1 ml. of molybdic acid solution were added to each liter of the undiluted solution. Other salts employed were:

NO_3/NH_4 ionic ratio of 100/0

KH_2PO_4	1.0 gram	$MgSO_4$		0.3 gram
$NaNO_3$	1.0 gram	$NaCl$		0.1 gram
$CaCl_2$	0.1 gram	Distilled H_2O		1000.0 ml.

Total = 0.25% solution by weight

NO_3/NH_4 ionic ratio of 50/50

KH_2PO_4	1.0 gram	$CaCl_2$		0.1 gram
Na_3PO_4	0.4 gram	$MgSO_4$		0.3 gram
NH_4NO_3	0.5 gram	$NaCl$		0.1 gram
Distilled H_2O	1000.0 ml.			

Total = 0.24% solution by weight

NO_3/NH_4 ionic ratio of 0/100

KH_2PO_4	1.0 gram	$(NH_4)_2SO_4$		0.75 gram
K_2HPO_4	0.2 gram	$MgSO_4$		0.3 gram
$CaCl_2$	0.1 gram	$NaCl$		0.1 gram

Total = 0.25% solution by weight

One liter of solution in each case contained 0.16 gram nitrogen. Three volumes of distilled water were added to one volume of solution for use. This gave a concentration of 0.06 per cent by weight with 0.004 per cent by weight of nitrogen.

Detmer's Solution

Three drops of 1 per cent aqueous solution of $FeCl_3$, 2 ml. of minor elements solution and 1 ml. of molybdic acid solution were added to a liter of the undiluted solution. The other salts are listed below.

NO_3/NH_4 ionic ratio of 100/0

$Ca(NO_3)_2$	1.0 gram	KH_2PO_4		0.25 gram
KCl	0.25 gram	Distilled H_2O		1000.0 ml.
$MgSO_4$	0.25 gram			

Total = 0.18% solution by weight

NO_3/NH_4 ionic ratio of 50/50

KH_2PO_4	0.25 gram	$(NH_4)_2SO_4$		0.4 gram
K_2HPO_4	0.15 gram	KCl		0.25 gram
$Ca(NO_3)_2$	0.5 gram	$MgSO_4$		0.25 gram
Distilled H_2O	1000.0 ml.			

Total = 0.18% solution by weight

NO_3/NH_4 ionic ratio of 0/100

KH_2PO_4	0.35 gram	$CaCl_2$		0.1 gram
K_2HPO_4	0.2 gram	KCl		0.25 gram
$(NH_4)_2SO_4$	0.6 gram	$MgSO_4$		0.25 gram
Distilled H_2O	1000.0 ml.			

Total = 0.18% solution by weight

One liter of either of the first two of these solutions contains 0.17 gram of nitrogen. The formula with a NO_3/NH_4 ionic ratio of 0/100 contains 0.13 gram per liter of nitrogen. For use two volumes of distilled water were added to one volume of solution. The culture medium then had a salt concentration of approximately 0.06 per cent by weight of which 0.006 per cent in the first two and 0.004 per cent in the third solution consisted of nitrogen.

Pringsheim's Solution

Two drops of 1 per cent aqueous solution of $FeCl_3$, 2 ml. of minor elements solution and 1 ml. of molybdic acid solution were added to a liter of undiluted solution. The composition of the solutions with the different ionic ratios follows:

NO_3/NH_4 ionic ratio of 100/0

KNO_3	1.0 gram	KH_2PO_4	0.2 gram
$MgSO_4 \cdot 7H_2O$	0.1 gram	Distilled H_2O	1000.0 ml.
$CaSO_4$	0.1 gram		

Total = 0.14% solution by weight

NO_3/NH_4 ionic ratio of 50/50

K_2HPO_4	0.2 gram	$MgSO_4 \cdot 7H_2O$	0.1 gram
KH_2PO_4	0.2 gram	$(NH_4)_2HPO_4$	0.3 gram
KNO_3	0.5 gram	$CaSO_4$	0.1 gram
		Distilled H_2O	1000.0 ml.

Total = 0.14% solution by weight

NO_3/NH_4 ionic ratio of 0/100

K_2HPO_4	0.2 gram	$(NH_4)_2HPO_4$	0.6 gram
KH_2PO_4	0.4 gram	$CaSO_4$	0.1 gram
$MgSO_4 \cdot 7H_2O$	0.1 gram	Distilled H_2O	1000.0 ml.

Total = 0.14% solution by weight

One liter of each of these solutions contained approximately 0.13 gram of nitrogen. For use seven volumes of water were added to three volumes of solution giving a concentration of 0.06 per cent by weight of nutrient salts in the diluted solution. Six-thousandths per cent by weight was nitrogen.

Tables 8 and 9 and figure 19 list data secured when solutions with different NO_3/NH_4 ionic ratios were used.

In all three solutions, plants supplied with nitrate nitrogen reached their greatest length and formed coenocysts more quickly than the others (fig. 27). Many such plants contained coenocysts after sixteen days.

Most plants on media with NO_3/NH_4 ionic ratio of 50/50 were in vegetative condition after twenty-four days (fig. 28). Plants with nitrate nitrogen were narrower in the rhizoidal portion than those with two nitrogen sources or with ammonium nitrogen only (figs. 27–29). Many of the plants that were supplied with ammonium nitrogen had bulb-like enlargements at the rhizoidal end (fig. 29). In all three solutions the longest sacs were produced on media with nitrate nitrogen (table 8, fig. 19). At the end of twenty-four days, the number of sacs in vegetative condition was much greater on

TABLE 8. *Effects on cell size of clone 8B2 of various culture media with different NO_3/NH_4 ionic ratios solidified with 1.5 per cent agar and grown at an average temperature of 17.5° C.*

Medium	NO_3/NH_4 ionic ratio	Mean length in mm. of 100 sacs after		
		8 days	16 days	24 days
Bristol's	100/0	0.14	0.47	0.70
	50/50	0.18	0.45	0.46
	0/100	0.13	0.26	0.26
Detmer's	100/0	0.32	0.50	0.90
	50/50	0.18	0.20	0.30
	0/100	0.13	0.19	0.30
Pringsheim's	100/0	0.16	0.54	0.76
	50/50	0.14	0.16	0.61
	0/100	0.17	0.21	0.28

media with low NO_3/NH_4 ionic ratios than it was on media with high ratios (figs. 27–30). Figure 19 shows that the results were similar for all three solutions.

TABLE 9. *Effects on the growth of clone 8B2 of various culture media with different NO_3/NH_4 ionic ratios used in liquid form at a temperature of 17.5° C.*

| Medium	NO_3/NH_4 ionic ratio	pH		Color intensity	Amount centrifuged from one flask (ml.)	Observations
		At beginning of experiment	After 36 days			
Bristol's	100/0	6.7	6.6	2 −	0.03	Gametes numerous. Most cells spherical
	50/50	6.6	6.4	2	0.07	More sacs than are usual in a liquid culture (fig. 31).
	0/100	6.1	6.0	2 −	0.04	Gametes numerous. Cells mostly spherical.
Detmer's	100/0	6.5	6.3	1	0.03	More dead cells than living ones.
	50/50	4.0	3.9	1	0.03	Cells spherical. Most of them dead.
	0/100	4.3	4.0	1	0.03	Cells spherical. Most of them dead.
Pringsheim's	100/0	6.1	6.3	2 −	0.03	Many cells with lobes separated by septa. Dead cells present but not as numerous as in Detmer's solution.
	50/50	6.6	6.7	0	0.01	Heavy precipitate present, some gametes and many dead cells.
	0/100	6.6	6.7	1	0.04	Cells mostly spherical. Many zygotes and many dead cells.

With Bristol's solution used in liquid form, growth was best when both nitrate and ammonium nitrogen were present. In two of the cultures based on Detmer's solution, the hydrogen-ion concentration was already too low for good growth when the inoculations were made. The precipitate that was present in Pringsheim's solutions with low NO_3/NH_4 ionic ratios may have removed some needed element from the solution. It is apparent that presence or absence of agar modifies the effect of varying NO_3/NH_4 ratios.

3. Effect of Minor Elements and Molybdenum. The quantity of minor elements required for growth of *Protosiphon* is almost certainly present in the form of unavoidable impurities in reagents even when rigorous care is used to exclude them from distilled water, nutrient salts, and from the culture vessels. Molybdenum is a recent addition to the supposed essential microelements. Steinberg (1936) showed that the decrease in yield of *Aspergillus niger* upon utilization of sucrose purified by alcohol was due almost entirely to the removal of zinc and molybdenum. Cultures were set up to determine whether the addition of micro-elements to solutions used for culture of *Protosiphon* would produce a measurable effect. Bristol's solution was used as the basic medium. The formulae for minor element solution (Craig & Trelease 1937) and for molybdic acid solution have been previously listed.

The longest sacs were obtained when 0.06 per cent Bristol's solution was used with 1 ml. of molybdic acid and 2 ml. of minor element solution per liter of undiluted medium. The amount of centrifugate obtained from this solution was three times as great as when Bristol's solution was used alone. Larger quantities of minor element solution were not beneficial, and the sacs produced were smaller with 4 ml. of minor element solution than with 2 ml. One ml. of molybdic solution gave better results than 2 ml. and the quantity of centrifugate was twice as great with the addition of this amount of molybdic acid as with Bristol's solution alone. Because of the results obtained in this experiment, 2 ml. of minor element solution and 1 ml. of molybdic acid solution were regularly added to a liter of undiluted Bristol's solution.

4. Hydrogen-Ion Concentration. The hydrogen-ion concentration of Bristol's solution when freshly prepared according to the original formula is pH 5.3. It can be lowered by substituting di-basic potassium phosphate for all or part of the mono-basic salt. For concentrations lower than pH 7.4, N NaOH or KOH was used. For hydrogen-ion concentrations higher than pH 5.3, 1.25 per cent phosphoric acid, N citric acid and N H_2SO_4 were used. Hydrogen-ion concentrations were adjusted in different ways in order to determine whether the effects produced were due to hydrogen or to other ions present.

It was found that growth was slower at first in cultures with alkaline reaction, but that these cultures remained green longer than those with acid
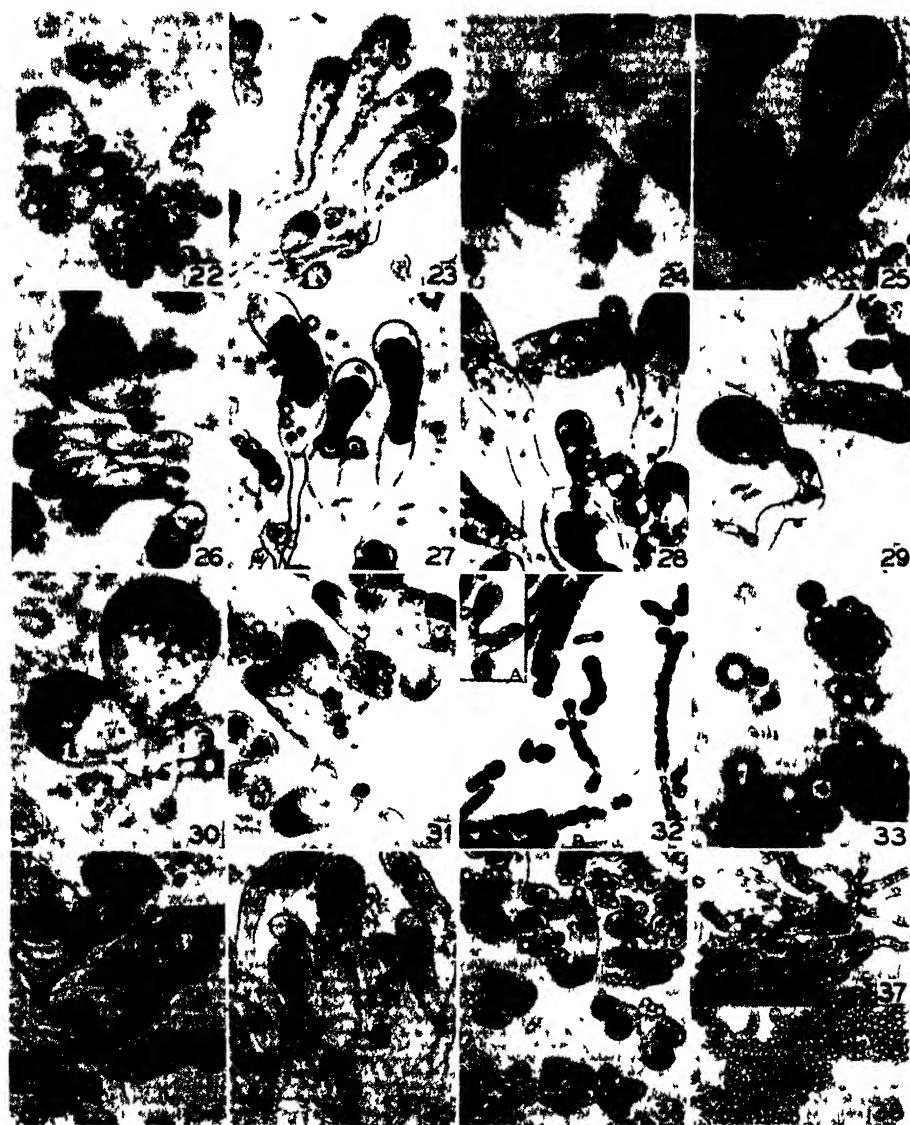
reaction. Microscopic examination showed that under the same conditions plants from alkaline cultures did not form coenocysts as soon as those from acid media (see also table 8). Evidence that growth continues longer in alkaline media was obtained when some flasks from a series with pH values ranging from pH 4.2 to pH 9.2 were centrifuged after 24 days growth and others from the same series were allowed to grow for 11 weeks. In flasks with an initial hydrogen-ion concentration of pH 4.2, the amount of centrifugate at the end of each period was 0.07 ml. At pH 5.1, the amount of centrifugate was 0.04 ml. at the end of 24 days and 0.08 ml. at the end of 11 weeks. At pH 8 4, the yield was 0.10 ml. after 24 days and 0.20 ml. after 11 weeks.

Another effect of alkaline media, especially noticeable in liquid cultures, is the inhibition of growth in the rhizoidal portion of the sacs. In figure 33, spherical cells from a liquid culture with pH 7.4 are filled with spherical aplanospores. In figures 31 and 36 thalli from liquid cultures with pH approximately 6.5 have developed rhizoidal portions.

Good growth was secured at pH 9.5 on both liquid and solid media. On the acid side, there was good growth on solid media at pH 3.4, but when this medium was used in liquid form, the liquid remained clear and the centrifugate contained only a few cells. This is further evidence of an increase in

Explanation of figures 22-38

Figs. 22-32, 34-38 100. Fig. 33 x 300. Fig. 22. Spherical cells from clone 8B2 growing for 24 days in 0.06% Bristol's solution (liquid). Fig. 23. Sacs about 0.75 mm. long from a culture of clone 8B2 on 0.06% Bristol's solution with 0.75% agar 24 days after inoculation. Fig. 24. Thalli from clone 8B2 grown for 24 days on 0.06% Bristol's solution with 4.5% agar. Fig. 25. Sacs 0.25-0.37 mm. long from clone 8B2 grown for 24 days on 0.06% Pringsheim's solution with 2% agar. Fig. 26. Sacs from 0.1-0.2 mm. long from clone 8B2 grown for 24 days on 0.06% Pringsheim's solution with 3% agar. Fig. 27. Sacs about 0.5 mm. long and tapering to a point from clone 8B2 grown 25 days on 0.06% Bristol's solution with an ionic ratio of 100 NO$_3$/O NH$_4$. Fig. 28. Vegetative sacs from clone 8B2 grown 24 days on 0.06% Bristol's solution with an ionic ratio of 50 NO$_3$/50 NH$_4$. Fig. 29. Sacs from clone 8B2 grown 24 days on 0.06% Bristol's solution with an ionic ratio of 0 NO$_3$/100 NH$_4$. The sac in the upper center of the figure is 0.25 mm. long and 0.04 mm. wide in the green part. Fig. 30. Sacs from clone 8B2 grown for 24 days on 0.06% Pringsheim's solution with an ionic ratio of 0 NO$_3$/100 NH$_4$. Fig. 31. Sacs from clone 8B2 grown for 36 days in 0.06% Bristol's solution (liquid) with an ionic ratio of 50 NO$_3$/50 NH$_4$. The sacs are from 0.12 mm. wide to 0.15 mm. long and from 0.03 mm. to 0.05 mm. in diameter. Fig. 32. A, Sac from clone 8B1 after 12 days growth in 0.06% Bristol's solution without Ca and solidified with 1.5% agar. B, Sacs from the same culture after 18 days with coenocysts filling the entire sac. Fig. 33. Spherical cells filled with growing aplanospores from clone 6B1 after 18 days growth in 0.06% Bristol's solution (liquid) with pH 7.4. Fig. 34. Sacs 0.3 mm. long from clone 8B2 grown for 24 days on 0.06% Bristol's solution – Ca and solidified with 1.5% agar. Fig. 35. Sacs 0.37 mm. long from clone 8B2 grown for 24 days on 0.06% Bristol's solution with 0.006% sodium oxalate and 1.5% agar. Fig. 36. Cells from clone 2B1 centrifuged from 0.06% Bristol's solution (liquid) with 0.5% fructose after 24 days growth. Fig. 37. Sacs from clone 2B1 grown 12 days on 0.06% Bristol's solution with 0.5% raffinose and 1.5% agar. Fig. 38. Sacs from clone 2B1 grown for 12 days on 0.06% Bristol's solution with 0.5% mannose and 1.5% agar.

growth when agar is added to the medium. At pH 4.2, there was good growth in liquid and 0.04 ml. of centrifugate was obtained from a flask after 18 days.

In twenty cultures, some with media in which the initial reaction was acid and others with alkaline media, the final hydrogen-ion concentrations were represented by pH values between 6 and 7. The optimum concentration for *Protosiphon* is probably within this range although good growth was secured in a much wider range.

5. Effect of Sodium Oxalate on the Growth of Protosiphon. Hopkins and Wann (1926, 1927) pointed out that iron can be almost completely removed from alkaline solutions by adsorption on calcium phosphate precipitate and that the lack of iron can be a limiting factor for growth of *Chlorella*. Inasmuch as many of the alkaline solutions used to test the effect of hydrogen-ion concentration contained precipitates a question arose concerning the possible effects of those precipitates.

Omission of calcium compounds lessened the difficulty with precipitates and apparently did not interfere with growth of the algae as determined quantitatively. One effect noted was that in one of the calcium-free cultures, there were odd-shaped sacs (fig. 32A) that lacked a rhizoidal portion. After eighteen days, the sacs contained coenocysts that were distributed throughout their lumens, and there was no clear rhizoidal portion (fig. 32B).

It seemed desirable to reduce the calcium content of the culture medium to a very low minimum in order to determine whether such a solution would support the growth of *Protosiphon*. Pringsheim (1926b) used concentrations of 0.006, 0.0125, 0.05, and 0.1 per cent sodium oxalate to precipitate calcium from nutrient solutions used to culture algae. He reported that algae which do not require calcium were not inhibited in their development by the presence of these concentrations of sodium oxalate, but he adds that traces of calcium sufficient for growth may be assimilated in the presence of oxalate.

Concentrations of sodium oxalate similar to those used by Pringsheim were added to modified, Bristol's solution with calcium omitted from the

TABLE 10. *Effect on the growth of clone 8B2 of 0.06 per cent Bristol's solution in 1.5 per cent agar without calcium and with the addition of sodium oxalate at 16.5° C.*

Medium	Mean length in mm. of 100 sacs after	
	12 days	24 days
0.06% B. (control)	0.45	0.45
0.06% B. – Ca (control)	0.35	0.44
0.06% B. + 0.006% $Na_2C_2O_4$	0.33	0.44
0.06% B. + 0.0125% $Na_2C_2O_4$	0.33	0.55
0.06% B. + 0.025% $Na_2C_2O_4$	0.19	0.26
0.06% B. + 0.05% $Na_2C_2O_4$	0.15	0.06
0.06% B. + 0.1% $Na_2C_2O_4$	0.16	0.06

formula in an attempt to precipitate as well all calcium present as impurities. Bristol's solutions with and without calcium were used as controls (table 10). All the solutions were perfectly clear both before and after sterilizing. It was observed that a visible precipitate was formed when 0.0005 per cent calcium chloride was added to a solution containing 0.0003 per cent sodium oxalate. The amount of calcium compounds present in the media must then have been less than 0.0005 per cent.

Sacs were smaller after 24 days than after 12 days on the cultures with 0.05 per cent and 0.1 per cent $Na_2C_2O_4$ because the sacs had continued to form gametes for a longer time than in some of the other cultures. In all the cultures, the plants were still in vegetative condition after twenty-four days (figs. 34, 35). On cultures with the higher concentrations of sodium oxalate, the smaller size of the cells appeared to be due to concentration rather than to a toxic effect of the salt.

In liquid cultures, the growth was good and the amount of centrifugate from flasks with 0.006 per cent sodium oxalate was greater than from any of the others not excepting the control cultures. The interpretation of these data is considered in the discussion.

B. Organic Media

1. Carbohydrates. In preliminary experiments it was observed that growth was stimulated by small quantities of glucose. Experiments were set up to determine the optimum concentration of glucose and to compare its effect with that of other carbohydrates on the growth of *Protosiphon*. Tables 11, 12 and figure 20 summarize data for effects on the growth of *Protosiphon* of concentrations of 0 25 and 0.5 per cent of a pentose and a hexose from the monosaccharide sugars, a disaccharide, and a polysaccharide, added to 0.06 per cent Bristol's solution.

Twenty-five-hundredths per cent dextrose produced more growth in liquid cultures and larger sacs on solid media than 0.5 per cent dextrose.

TABLE 11. *Effects of certain sugars added to 0.06 per cent Bristol's solution with 1.5 per cent agar on the growth of clone 2B1 at a temperature of 20.5° C.*

Carbohydrate	Average length in mm. of 100 sacs after			
	6 days	12 days	18 days	24 days
0.25% dextrose	0.03	0.23	0.47	0.54
0.5% dextrose	0.04	0.26	0.30	0.32
0.25% inulin	0.03	0.18	0.22	0.25
0.5% inulin	0.01	0.47	0.47	0.75
0.25% sucrose	0.04	0.18	0.30	0.30
0.5% sucrose	0.03	0.32	0.37	0.43
0.25% xylose	0.01	0.07	0.14	0.14
0.5% xylose	0 02	0.03	0.03	0.10

This observation as well as some made in other experiments indicates that the optimum concentration of dextrose may be less than 0.25 per cent. Five-tenths per cent inulin produced the largest sacs on solid media and the greatest amount of centrifugate in liquid media. When it is used in liquid media, the results are very noticeable and large well developed sacs are found instead of the spherical ones that usually predominate in liquid media. Although in the present experiment larger sacs were produced on solid media

TABLE 12. *Effects on the growth of clone 2B1 of certain sugars added to 0.06 per cent Bristol's solution (liquid form) at 20.5° C.*

Amount of sugar	pH		Color intensity	Volume centrifuged from one flask (ml.)	Observations
	At beginning of experiment	After 24 days			
0.25% dextrose	6.0	6.6	4	0.055	Small cells with one or two coenocysts.
0.5% dextrose	6.0	6.6	4 –	0.07	Small cells with one or two coenocysts.
0.25% inulin	6.2	7.0	4 –	0.09	Sacs 0.12 mm. long in vegetative condition. Many zygotes.
0.5% inulin	6.2	7.0	4	**0.12**	Sacs 0.35 mm. long in vegetative condition.
0.25% sucrose	6.3	6.4	2	0.05	Small cells with single coenocysts 0.02 mm. in diameter.
0.5% sucrose	6.3	6.4	2 –	0.03	Small cells with single coenocysts 0.02 mm. in diameter.
0.25% xylose	5.1	5.9	3	0.06	Sacs 0.12 mm. long most of them in vegetative condition.
0.5% xylose	5.2	5.8	1	0.01	More dead than living cells. Some healthy looking sacs 0.1 mm. long.

with 0.5 per cent sucrose than with 0.25 per cent sucrose, the results obtained from liquid cultures and from other experiments indicate that the lower concentration of sucrose is more effective in stimulating growth of *Protosiphon*.

An experiment with higher concentrations of the same sugars shown in tables 11 and 12 gave further indication that higher concentrations are less effective than lower ones. Sacs in 1 per cent inulin were a little longer than they were in 0.5 per cent inulin, but inasmuch as this carbohydrate dissolves with difficulty in cold water and there was a ring of precipitate at the surface of the liquid in the flasks, it was apparent that the amount of inulin in the solution was less than 1 per cent.

Because equal concentrations of the carbohydrates used in the foregoing experiment did not react in the same way, it seemed desirable to use others and compare their effects on the growth of *Protosiphon*. Table 13 and figure 21 present data for the results obtained with the addition of two types of monosaccharides, a disaccharide, and a trisaccharide to 0.06 per cent Bristol's solution used to culture *Protosiphon*.

TABLE 13. *Effect of the addition of certain sugars to 0.06 per cent Bristol's solution with 1.5 per cent agar on the length of sacs of clone 2B1 at a temperature of 20° C.*

Concentration of sugar	Mean length in mm. of 100 sacs after			
	6 days	12 days	18 days	24 days
0.25% fructose	0.01	0.13	**0.55**	0.55
0.5% fructose	0.03	0.08	0.12	**0.75**
0.25% maltose	**0.08**	0.22	0.25	0.25
0.5% maltose	0.05	0.18	0.21	0.31
0.25% mannose	0.01	0.03	0.18	0.20
0.5% mannose	0.01	0.05	0.31	0.55
0.25% raffinose	0.01	0.19	0.25	0.33
0.5% raffinose	0.03	**0.26**	0.26	0.29

In this group of sugars, fructose gave the most growth and the longest sacs at the end of 24 days. Sacs on medium with 0 5 per cent mannose were much longer than those on the same concentration of maltose and raffinose, although for the first twelve days growth on mannose was slower than on either raffinose or maltose (figs. 37, 38). In liquid cultures, the greatest amount of centrifugate was obtained from media with mannose and fructose. Many small sacs with lobes separated by septa were observed in the liquid cultures with 0.5 per cent fructose. (fig. 36).

2. Proteins. Six flasks of beef extract (beef extract, 3 grams; sodium chloride, 5 grams; peptone, 10 grams; distilled water, 1000 ml.) diluted to a concentration of 0.06 per cent by weight were inoculated with clone 3B1. Growth was very good and appeared to be concentrated at the bottom of the flasks in contrast to the heavy surface rings when sugars were used.

Good growth was also secured in solution with no nutrient but peptone. A dilution of 0.04 per cent gave better results than either 0.02 or 0.08 per cent peptone dilutions. A solution containing peptone and 0.06 per cent Detmer's solution also produced good growth of *Protosiphon*.

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STUDIES IN THE CARYOPHYLLACEAE—III. A SYNOPSIS OF THE NORTH AMERICAN SPECIES OF ARENARIA, SECT. EREMOGONE FENZL

BASSETT MAGUIRE

The following is an enumeration of the North American species of *Arenaria*, all perennial, bearing narrowly linear and pungent or setaceous leaves, flower-discs that are provided with more or less conspicuous glands at the base of, and externally affixed to the stamens, and firm-walled capsules dehiscing by 6 teeth. In most species of this group, the antisepalous gland is conspicuously enlarged, entire or bilobed, and the alternate glands inconspicuous or obsolete. Less often are the ten glands of equal development, or altogether obsolete.

There is little doubt that American species falling into the characterization given above are closely interrelated and must be interpreted as belonging to a single natural section, notwithstanding the contrary disposition by the eminent Caryophyllologist, F. N. Williams.[1] Critical examination of an abundance of American material fails to support the segregation of species with enlarged antisepalous glands into the subgenus *Pentadenaria*. Indeed this condition obtains in most of the American species assigned by Williams (and essentially so maintained by Pax and Hoffman[2]) to both the subgenera *Eremogone* and *Pentadenaria*, and the character must be considered, at least with our species, of questionable value as determinant for subgeneric or sectional segregation.

Relationships among members of the American *Capillaris*-group are complex and obscure. Within it little clear-cut specific differentiation has taken place, or at least most species are so variable within themselves as to transgress all specific lines, removing discontinuity, and to leave only emphatic nodes in a basally almost continuous series.

If we consider *A. capillaris*, in the major western hemisphere representation the boreal subsp. *americana*, as the primitive and deriving member of the group—a position logically to be taken because of the chiefly whole-arctic distribution of the genus, and morphological mergence of the subspecies with austral presumptive derivatives—three principal lines of differentiation have taken place, these well correlated with geographic divergence and relative isolation.

1. Most directly differentiation has been made into *A. aculeata* and the

[1] A Revision of the genus Arenaria Linn. Jour. Linn. Soc. **33**: 334. 1898.
[2] Caryophyllaceae in Engler & Prantl, Nat. Pflanzenf. **16C**: 316. 1934.

more poorly segregated *A. pumicola, A. aberrans,* and *A. ursina,* all held together by obtuse hyaline-margined sepals, open inflorescence, and general habit.

2. The subsp. *americana* merges into *A. Kingii* where their ranges are coincident; into the subsp. *Kingii* in northern Nevada and southeastern Oregon, and into the subsp. *uintahensis* in northern Utah. *A. macradenia* seems to be a southern outgrowth of *A. Kingii*—possibly through the var. *glabrescens.*

3. The *A. congesta–A. Fendleri* complex   Habitally these two species resemble the subsp. *americana,* but have differentiated from their precursor and between themselves in the achievement of a congested inflorescence on the one hand and acuminate-attenuate sepals on the other. *A. Eastwoodiae* is an obvious Colorado Plateau derivative of *A. Fendleri.* The far disjunct desert range *A. stenomeres,* on morphological grounds, chiefly acuminate-attenuate sepals, seems to be related to *A. Fendleri.*

The position of *A. Hookeri* and *A. Franklinii* is obscure. While they both have developed cephaloid inflorescences, in habit they more resemble *A. Nuttallii* Pax, of the section *Alsine.*

The accompanying chart suggests diagrammatically the possible relationships and lines of derivation of the several species in the *Capillaris*-group.

A. Franklinii   A. Hookeri

A macradenia   A aberrans   A pumicola   A.ursina   A. Eastwoodiae   A.stenomeres

A Kingii · · ·· A aculeata ········ ··· A. congesta   A. Fendleri

A.capillaris americana
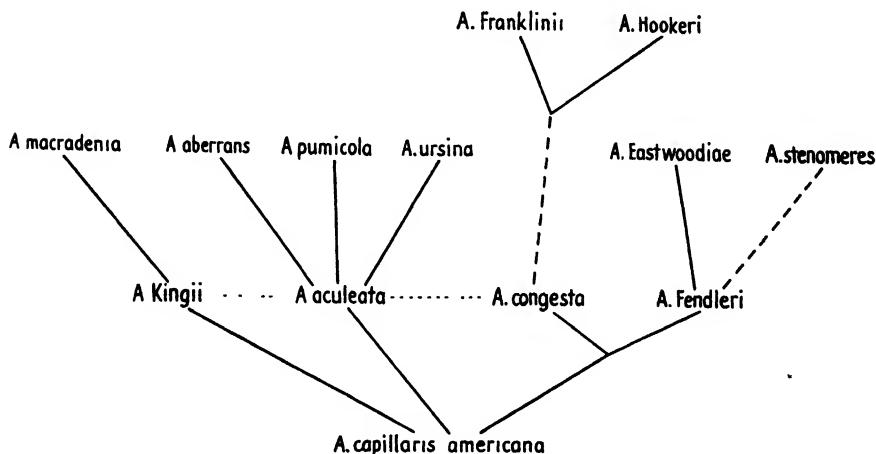
Study in the Caryophyllaceae has inevitably been attended by protests of weak characterization in the family. Certainly this condition obtains for the group here under consideration. "Critical" specific characters are feeble. The interpretation of populations, specific and subspecific, depends largely on the accurate evaluation of the interplay of sepal, leaf, and inflorescence form. Gland size and disposition, seed, and rootstock offer supporting characterization. Of no little aid is the final summation of intangibles that comes of familiarity with the plants, and that give populations an "appearance"

or "look"—indefinite properties that defy descriptive analysis. Considerable
weight has been also given to segregation from correlatable natural ranges.
The weight attached to and the importance of all of these criteria is evident
from the body of the paper.

SECTION EREMOGONE FENZL IN ENDL. GEN. 1: 967. 1840.

Sect. *Pentadenaria* Williams, Bull. Herb. Boiss. **3**: 598 (at least as to
American species). 1895. Subg. *Pentadenaria* Williams, Jour. Linn. Soc.
33: 334 (at least as to American species). 1898. Subg. *Euarenaria* sect.
Eremogoneae Williams, Jour. Linn. Soc. **33**: 338 (at least as to American
species). 1898.

Flower-disc with more or less prominent glands at the base and exter-
nally adnate to the 10 stamens, the antisepalar glands usually more promi-
nent and the antipetalar glands inconspicuous or obsolete, or all glands
inconspicuous, even sometimes essentially obsolete; sepals more or less in-
durate at the base; capsule more or less indurate, partially dehiscing along
the commissural sutures, each valve-apex in turn bidentate; leaves narrowly
linear and more or less pungent, setaceous or subulate; perennial, frequently
suffrutescent.

KEY TO THE SPECIES

1. Sepals obtuse, or merely acutish or apiculate.
   2. Inflorescence open (at least at full maturity), more or less dichoto
      mous.
      3. Leaves recurved or flexuous, mostly (except in alpine forms) more
         than 2 cm. long; stems frequently leafy; seed 2.0–2.5 mm. long.
                                                      1. *A. capillaris americana.*
      3. Leaves aculeate or ascending, pungent, or fleshy, essentially basal,
         mostly 2 cm. or less long; seed 2.5–3.0 mm. long.
         4. Leaves stiffly pungent; plants characteristically glaucous and
            matted.   2. *A. aculeata.*
         4. Leaves more or less fleshy or not stiffly pungent; plants usually
            neither glaucous nor matted.
            5. Leaves strictly ascending, sepals 3–4 mm. long, capsules 4.5–5.5
               mm. long.   3. *A. pumicola.*
            5. Leaves aculeate or recurved.
               6. Leaves aculeate, 1–2 cm. long; sepals 4–5 mm. long, capsules
                  7–9 mm. long.   4. *A. aberrans.*
             ' 6. Leaves more or less recurved, 0.5–1.0 (1.5) cm. long; sepals
                  3–4.5 mm. long, capsules 3–5 mm. long.   5. *A. ursina.*
   2. Inflorescence congested, subcongested, or umbellate.   6. *A. congesta.*
1. Sepals acute to acuminate.
   2. Inflorescence open (at least at full maturity), more or less dichoto-
      mous.
      3. Sepals narrowly acute to acuminate.
         4. Petals broadly oblong-elliptic to oblanceolate, entire, retuse or
            somewhat erose; glands obvious or conspicuous; plants of the
            Rocky Mountain and Colorado Plateau.
            5. Stems glandular-puberulent, with 5 or more pairs of leaves;
               glands oval-oblong, ca. 0.5 mm. long; plants of the southern
               Rocky Mountain and high Colorado Plateau.   7. *A. Fendleri.*
            5. Stems glabrous or glandular, leaves essentially basal; glands
               oblong-truncate, 1–2 mm. long; plants of desert regions,
               Colorado Plateau.   8. *A. Eastwoodiae.*

4. Petals linear, emarginate, glands minute or obsolete; plants of the
   Great Basin.   11. *A. stenomeres.*
3. Sepals broadly acute.
   4. Stems mostly 2–4 dm. tall and woody at the base; sepals 4.5–6.0
      (6.6) mm. long; petals entire, erose, or retuse; glands 1–2 mm.
      long; plants of the Mohave Desert and lower Great Basin.   9. *A. macradenia.*
   4. Stems mostly 2 dm. or less tall and not woody at the base; sepals
      3.6–4.5 (6.0) mm. long; petals entire, erose, retuse, or cleft
      nearly to base; glands ca. 0.5 mm. long; plants of the Great
      Basin.   10. *A. Kingii.*
2. Inflorescence congested, subcongested, or proliferated.
   3. Sepals 3–5 (6) mm. long, acute.   6. *A. congesta.*
   3. Sepals (5.5) 6–15 mm. long. acuminate.
      4. Stems leafy, glabrous; sepals 8.5–15 mm. long (5–6 mm. long in
         var. *Thompsoni*); dry slopes and plains of eastern Oregon and
         Washington.   12. *A. Franklinii.*
      4. Stems not leafy, scabrid-puberulent; sepals 5.5–8.0 mm. long;
         plains and barren slopes and ridges, mostly east of the con-
         tinental divide; Elko County, Nevada.   13. *A. Hookeri.*

1. ARENARIA CAPILLARIS Poir. Encycl. Meth. **6**: 380. 1804.

KEY TO THE SUBSPECIES

Sepals usually 5–6 mm. long; (in ours) totally glabrous.   *A. capillaris* subsp. *capillaris.*
Sepals usually 4.0–4.5 mm. long, inflorescence glandular. *A. capillaris* subsp. *americana.*

ARENARIA CAPILLARIS Poir. subsp. **capillaris** Maguire, subsp. nov.

A. capillaris Poir. Encycl. Meth. **6**: 380, as to type. 1804.
A. formosa Fisch. ex DC. Prodr. **1**: 402. 1824.
A. nardifolia Ledeb. Fl. Altaic **2**: 166. 1830.
   A. capillaris Poir. Grex c., ε. *formosa* (Fisch.) F. N. Wms. Jour. Linn. Soc. **33**: 415,
exclusive of American plants. 1898.
   A. capillaris Poir. Grex c., ζ. *nardifolia* (Ledeb.) F. N. Wms. Jour. Linn. Soc. **33**:
415. 1898.

Occurring sparingly in Alaska, differing from subsp. *americana* in its
larger flowers and total glabrousness. Highly polymorphous, this extensive
population has frequently been the object of extensive redivision. The Alas-
kan specimens are probably to be referred to the var. *nardifolia* (Ledeb.)
Regel.

Type locality: "Cette plante a été recueillie par M. Patrin dans la
Sibérie."

Distribution: Asia from the Angara-Sagan District (fide Hultén) to
Alaska. Reported from the Northwest Territory (*A. nardifolia*) by Porsild.

Representative specimens: Healy, Alaska, July 17, 1922, *Anderson 1624* [var. *nardi-
folia* (Ledeb.) Regel].

ARENARIA CAPILLARIS Poir. subsp. **americana** Maguire, subsp. nov.

A. capillaris of American authors, not *A. capillaris* Poir.
A. nardifolia of American authors, not *A. nardifolia* Ledeb.
A. formosa of American authors, not *A. formosa* Fisch.
   A. capillaris Poir. subsp. *formosa* (Fisch.) Maguire, Madroño **6**: 24. 1941. Not *A.
formosa* Fisch.

Caulibus (3) 5–15 (25) cm. longis, foliis lineari-subulatis, acutis, serrulatissimis, glabratis vel glanduloso-puberulentibus, foliis caulinis 2-jugis aliquando frondosis; inflorescentibus non congestis, rare subcongestis; pedicellis (2) 5–10 (15) mm. longis; sepalis (3) 3.5–4.5 (4.8) mm. longis; petalis (5) 6–10 mm. longis, late vel anguste elliptico-oblanceolatis; glandis ad 0.4 mm. longis; capsulis ovatis, ad apices fere granulari-pubescentibus, seminibus 2.0–2.5 mm. longis, non marginatis.

The American race may be recognized by its consistently smaller flowers; in subsp. *americana* the usual and average calyx length being 4.2 mm. with extreme range of 3.0–4.8 mm.; whereas the Asiatic races, including *A. formosa* Fisch. and *A. nardifolia* Ledeb. have the usual and average calyx length of 5.4 mm., the extremes at 3.6–6.6 mm., very few specimens having the shorter measurements. To the south of its range the subsp. *americana* is poorly separated from *A. aculeata* and *A. Kingii.*

The lower montane (and southern) form tends to be somewhat taller and to have somewhat larger flowers with more acutish sepals than boreal or alpine plants.

Type locality: Hurricane Ridge, Olympic Mountains, Clallam County, Washington, *J. W. Thompson 14162* (N. Y. Bot. Gard.).

Distribution: Mountains and plains, British Columbia and Alberta south in the mountains to central Oregon, Idaho, Montana, and northwestern Nevada. Possibly in Alaska.

Representative specimens: Skagit River, B. C., July 22, 1905, *J. M. Macoun* (Geol. Surv. Cana. 79578); Banff, Alberta, July 7, 1899, *W. C. McCalla 2410*; Challis, Custer County, Idaho, June 15, 1944, *Hitchcock & Muhlick 8976*; Wiessner Peak, Kootenai County, Idaho, July 8, 1892, *Sandberg et al. 589*; Ole Creek, Glacier National Park, Montana, June 30, 1934, *Maguire & Piranian 15711*; Mt. Stuart, Missoula, Montana, July 3, 1921, *Kirkwood 1105*; Moffats Diamond A Ranch, Elko County, Nevada, May 29, 1939, *York* sine no.; Virden, Kittitas County, Washington, June 8, 1935, *Thompson 11580*; Mt. Paddo, Washington, August 11, 1909, *Suksdorf* sine no.; Mt. Hood, Oregon, August 27, 1905, *Lyon 161.*

2. ARENARIA ACULEATA S. Wats. Bot. King's Expl. **5**: 40. 1871. Not *A. aculeata* Desv. Jour. de Bot. **3**: 221. 1816 (nomen nudum).

A. *salmonensis* Henderson, Bull. Torrey Club 27: 343. 1900.

Type locality: Fremont's Pass, East Humboldt (Ruby) Range, Elko County, 6500 feet, in fruit, August, 1868, *S. Watson 170* (Gray Herb.).

Distribution: Dry gravelly slopes, from 6000 to 10,000 feet elevation; Beaverhead County, Montana, Custer, Blaine and Valley Counties, Idaho, to Wallowa and Baker Counties, Oregon, south to Modoc and Plumas Counties, California, northern Nevada, and Box Elder County, Utah.

Representative specimens: Anthony Lake Region, Blue Mountains, Baker County, Oregon, July 23, 1936, *Thompson 13444*; Crane Mountain, Lake County, Oregon, July 12, 1936, *Thompson 13238*; Strawberry Mountains, Grant County, Oregon, July 15, 1921, *Peck 10261*; Warner Mountains, Modoc County, California, August 8, 1935, *Wheeler 3809*; Sawtooth Mountains, Blaine County, Idaho, August 8–11, 1939, *Hitchcock & Martin 5730*; Bonanza, Custer County, Idaho, July 25, 1916, *McBride & Payson 3443*; Santa Rosa Range, Humboldt County, Nevada, July 22, 1937, *Train 496*; Lamoille Canyon, Ruby Range, Elko County, Nevada, July 22, 1945, *Maguire & Holmgren 22057*; Dunn Canyon, Raft River Mountains, Box Elder County, Utah, July 31, 1943, *Maguire & Holmgren 22198.*

3. ARENARIA PUMICOLA Coville and Leiberg, Proc. Biol. Soc. Wash. **11**: 169. 1897.

KEY TO THE VARIETIES

Leaves fleshy; stems hardly ligneous at the base.   1. *A. pumicola* var. *pumicola*.
Leaves not fleshy; stems ligneous at the base.   2. *A. pumicola* var. *californica*.

ARENARIA PUMICOLA var. **pumicola** Maguire, var. nov.

A. pumicola Coville and Leiberg, Proc. Biol. Soc. Wash. **11**: 169, as to type. 1897.

A. pumicola is an unsatisfactorily distinct species and may finally have to be interpreted as a part of *A. aculeata,* as a subspecific or even varietal ecologic population.

Type locality: Pulverized pumice-stone, rim Crater Lake, altitude 2180 m., Klamath County, Oregon, August 13, 1896, *Coville and Leiberg 349* (U. S. Nat. Herb.).

Distribution: Apparently restricted to loose pumice, Klamath County, Oregon. Plants intermediate to the next are known from Josephine County, Oregon.

Representative specimens: Crater Lake, Oregon: *Cusick 2977*; *Heller 13457*; *Maguire et al. 15095*. Abbott Butte, Jackson County, Oregon, July 2, 1936, *Thompson 13050*, is intermediate to the var. *californica.*

ARLNARIA PUMICOLA var. **californica** Maguire, var. nov.   Caudice ligneo, caulibus 15–30 cm. altis; foliis vix succulentis, foliis caulinibus, non basilaribus.

As circumscribed here, the var. *californica* is not a strong natural population, but is set up about three dispersed collections, more woody and vigorous, and less fleshy than the typical race.

Type locality: Lake Valley, Lake Tahoe Region, California, July 27, 1911, *LeRoy Abrams 4779* (N. Y. Bot. Gard.).

In addition to the type, the following two collections are known to me: Lover's Leap, Horsetail Falls trail, El Dorado County, California, August 12, 1936, *Belshaw 2628;* "California," 1875, *Lemmon* sine no.

4. ARENARIA ABERRANS M. E. Jones, Contr. West. Bot. **16**: 37. 1930.

A. Rusbyi Greene, in Heller, Cat. N. Am. Pl. ed. 1. 49. 1898 (nomen nudum).

This species is a good, clear segregate closely derived from *A. capillaris* and related to *A. aculeata* and *A. pumicola,* but is sharply set off by its fleshy basally disposed leaves, large flowers, and relatively immense capsules.

Type locality: Dry hills, oak assn., 7000 ft. elevation, Mt. Dellanbough, Mohave County, Arizona, *Cottam 1159,* (Pomona; isotype at Brigham Young University).

Distribution: Apparently restricted to oak and yellow pine areas from 5500–9000 ft., Colorado and Kiabab Plateaus, Gila, Coconino, and Mohave Counties, Arizona.

Representative specimens: Hollow Canyon, Arizona, May 17, 1883, *Rusby 532*; Grand Canyon, Arizona, June 26, 1898, *McDougal 169;* between Payson and East Verde Bridge, Arizona, May 19, 1935, *Nelson & Nelson 1999*; South Gate, Grand Canyon, Coconino County, Arizona, June 27, 1935, *Maguire 12236.*

5. ARENARIA URSINA Robins. Proc. Am. Acad. **29**: 294. 1894.

Arenaria capillaris var. *ursina* (Robins.) Robins. Syn. Fl. N. Am. **1**: 240. 1897.

Not so clearly a distinct derivative as the above from its *A. capillaris*.

Type locality: Dry hills, San Bernardino Valley, San Bernardino Mountains, Bernardino County, California, August, 1882, *S. B. & W. F. Parish* sine no. (Gray Herb.).

Distribution: Dry hills and slopes 7000–10,000 ft., San Bernardino, Inyo, Fresno, and Riverside Counties, California; possibly the range more extended.

Representative collections: Bear Valley, San Bernardino County, California, June 25, 1894, *Parish 3124*; Grapevine Mountain, California, June 9, 1891, *Coville & Funston 1764*; Black Mountain, Fresno County, California, July, 1900, *Hall & Chandler 591*.

6. ARENARIA CONGESTA Nutt. ex Torr. & Gray, Fl. N. Am. 1: 178. 1838.

Perhaps the most widely distributed and polymorphous species in the *Capillaris*-group, *A congesta* is here interpreted as, in addition to the more extensive typical population, having developed ten more or less distinctive and partially geographically restricted variants.

The chief characters about which variation takes place, and upon which segregation has been made, is the degree of congestion of the inflorescence, form of the sepal apex, and to some extent the form and disposition of the leaves. Only the varieties *charlestonensis* and *crassula* depart from the important general habit of the species in having short basally disposed leaves. The theme of variation otherwise plays upon a proliferation, or better, a relaxation of the inflorescence, and the sepals becoming acute or acuminate as against the more prevalent obtuse condition. Neither of these modifications appears to be linked, but may appear independently or coincidentally.

Effort was made to separate the Sierran plants with umbellate inflorescence as a subspecies, distinct from the northern and eastern members of the species. This attempt was given up, however, since the typical form occurs concomitantly with it, and its character tends to appear throughout the general range of the species.

The most tenuous entity recognized is the var. *subcongesta*, wherein complete intermediacy is accomplished between *A. congesta* and the almost equally polymorphous *A. Kingii*. Because of this, however, no advantage can be seen in attempting to link the two populations specifically. To do so would necessitate sweeping together many of the more fluid species, viz., *A. congesta*, *A. Kingii*, and *A. capillaris* and derivatives, even perhaps *A. Fendleri*.

KEY TO THE VARIETIES

1. Sepals obtuse.
  2. Inflorescence compactly congested.
    3. Leaves 3–6 (8) cm. long, filiform.   *A. congesta* var. *congesta.*
    3. Leaves 2–3 cm. long, 1–2 mm. wide, crassulus and subsucculent.
                             A. congesta var. *crassula.*
  2. Inflorescence proliferating or umbellate.
    3. Inflorescence proliferating, forming more or less loose, irregular cymes; sepals 4–5 mm. long; central Idaho and adjacent Montana and Wyoming, probably also in Colorado and Utah.   *A. congesta* var. *expansa.*
    3. Inflorescence umbellate, the bracts arising approximately at the summit of the peduncle; sepals 3–4 mm. long; Sierra Nevada.
                          A. congesta var. *suffrutescens.*

1. Sepals acute.
   2. Inflorescence compactly congested, the flowers essentially sessile.
      3. Leaves filiform, 3–8 cm. long; plants of central Idaho and adjacent
         Montana and Wyoming.   *A. congesta* var. *cephaloidea.*
      3. Leaves not filiform, 1–2 cm. long, less than 1 mm. broad; leaves and
         sepals pungent; Charleston Mountains, Nevada.
                              A. congesta var. *charlestonensis.*
   2. Inflorescence proliferating, or the flowers obviously pedicelled.
      3. Stems 15–20 cm. or more high, leafy; leaves (2) 3–8 cm. long, fili-
         form, usually strict; dry slopes east of Cascade Divide, Oregon and
         Washington.   *A. congesta* var. *prolifera.*
      3. Stems usually 15 cm. or less high, not leafy; leaves 2 cm. or less
         long, broader, usually divergent or recurved.
         4. Inflorescence usually little congested; sepals 4.0–5.0 (5.5) mm.
            long, weakly or not at all 3 nerved; dry slopes east of the Sierra
            Nevada; Great Basin.   *A. congesta* var. *subcongesta.*
         4. Inflorescence usually more or less closely congested; sepals (5.0)
            5.5–6.5 mm. long.
            5. Stems 5–10 cm. high; leaves usually strongly recurved; sepals
               conspicuously 3 nerved; Snake Range, White Pine County,
               Nevada.   *A. congesta* var. *wheelerensis.*
            5. Stems 10–15 cm. high; leaves usually strict; sepals feebly or not
               at all 3 nerved; plants of Lassen and Modoc Counties, Cali-
               fornia, and adjacent Nevada.   *A. congesta* var. *simulans.*

ARENARIA CONGESTA var. **congesta** Maguire, var. nov.

A. congesta Nutt. ex T. & G. Fl. N. Am. 1: 178, as to the type and most of the population. 1838.

Type locality: "Shady hills in the Rocky Mountain range, about Bear River of the Lake of Timpanogos."

Distribution: The most widespread assemblage within the species; plains and mountain slopes and ridges to 11,500 ft.; Washington and Montana to central California, and southern Nevada, Utah, and Colorado.

ARENARIA CONGESTA var. **crassula** Maguire, var. nov.   Caulibus 15–20 cm. altis; foliis 1.5–3.0 cm. longis, 1–2 mm. latis, crassulis, subsucculentibus, basilaribus, inflorescentibus dense congestis; sepalis 3.5–4.0 mm. longis, obtusis, crassulis, subsucculentibus.

Type locality: Alpine slopes of Ashland Peak, 7500 ft., Siskiyou Mountains, Jackson County, Oregon, July 28, 1935, *Thompson 12358* (N. Y. Bot. Gard.). Striking in character; its habit, with the exception of the dense spheroid head and broad subsucculent leaves, is that of *A. pumicola*, suggesting a possible hybrid origin between *A. congesta* var. *congesta* and *A. pumicola*

Distribution: Upper montane slopes, Siskiyou Mountains, southwestern Oregon and adjacent California.

Representative specimens: Marble Mountains, Siskiyou County, California: July 9, 1939, *Hitchcock & Martin 5324*; June 1901, *Chandler 1674*; Devil's Garden, September 12, 1935, Modoc County, California, *Wheeler 3980* (flowers large).

ARENARIA CONGESTA var. **expansa** Maguire, var. nov.   Caulibus 15–30 cm. altis; foliis basilaribus filiformibus, 3–6 cm. longis; inflorescentibus pro-liferatis; sepalis 3–4 mm. longis, obtusis.

In habit like the varieties *congesta* and *prolifera,* but differing from the former by its open inflorescence, and from the latter by its smaller and obtuse sepals.

Type locality: Rocky granitic slopes near Wildhorse Creek, 25 miles S.W. Chilly, Custer County, Idaho, 7300 ft., July 22, 1941, *A. Cronquist 3309* (N. Y. Bot. Gard.). Cotype: north exposure, small canyon 4 miles N.E. Dickey, Custer County, Idaho, 7800 ft., July 15, 1941, *A. Cronquist 3137* (Intermountain Herb.).

Distribution: Central Idaho and adjacent Beaverhead County, Montana, and Yellowstone Park, Wyoming, perhaps more widely spread.

Representative specimens: Yellowstone National Park, Wyoming, *Maguire & Maguire 1157*; Gibbonsville, Lemhi County, Idaho, *Hitchcock et al. 3695.*

ARENARIA CONGESTA var. SUFFRUTESCENS (Gray) Robins.

Brewerina suffrutescens Gray, Proc. Am. Acad. 8: 620, as to type. 1873.
Arenaria congesta Nutt. ex T. & G. var. *suffrutescens* (Gray) Robins. Proc. Am. Acad. 29: 295. 1894.
A. suffrutescens (Gray) Heller, Muhlenbergia 6: 96. 1910.

Within the var. *suffrutescens* two interesting forms occur: from Placer County south the population has glandular stems and more strict leaves, while to the northward from Plumas County to Josephine County, Oregon, the plants consistently have glabrous stems and longer leaves tending to become flexuous. If the population with umbellate inflorescence could be treated as a subspecies, these forms of obvious taxonomic significance would fall as varieties.

Type locality: Sierra Nevada above Cisco (and between Truckee and Donner Lake), Placer County, California, *Bolander & Kellogg* (Gray Herb.).

Distribution: Mountain slopes 5300 to 11,000 ft., Siskiyou Mountains and the Sierra Nevada Range, from Josephine County, Oregon, south to Tulare County, California.

Representative specimens: Pubescent form: Emigrant Gap (Placer County), California, May 28, 1882, *Jones 3278*; Emigrant Gap, June 19, 1917, *Heller 12741*; Hockett's Meadow, Tulare County, California, July 22, 1904, *Baker 4476*. Glabrous form: Genesee Valley, Plumas County, California, July 16, 1907, *Heller & Kennedy 8869*; Mt. Eddy, Siskiyou County, California, August 18, 1903, *Baker 3908*; Lake Mountain, Siskiyou Mountains, Josephine County, Oregon, July 7, 1939, *Hitchcock & Martin 5140.*

ARENARIA CONGESTA var. **cephaloidea** (Rydb.) Maguire, comb. nov.

A. cephaloidea Rydb. Bull. Torrey Club 39: 316. 1912.

Similar in habit to var *congesta,* but with lanceolate, acute, and somewhat pungent sepals.

Type locality: Open gravelly wood, Spokane County, Washington, August 10, 1902, *Kreager 617* (N. Y. Bot. Gard.).

Distribution: Essentially confined to central and northwestern Washington and adjacent Idaho, this variant has not been frequently collected. Reported from the Uinta Mountains, Utah, by Goodman (Ann. Mo. Bot. Gard. 18: 284. 1931).

Representative specimens: Little Potlatch River, Latah County, Idaho, June 2, 1892, *Sandberg et al. 478*, cotype; dry prairies, Spokane County, Washington, June 27, 1884, *Suksdorf* sine no., cotype; St. Mary's River, northern Idaho, July 4, 1895, *Leiberg 1164*; Boise, Boise County, Idaho, June 2, 1944, *Hitchcock & Muhlick 8640.*

ARENARIA CONGESTA var. CHARLESTONENSIS Maguire, Bull. Torrey Club
72: 326. 1946.

Type locality: Harris Spring Summit Road, 18 miles south Kyle Canyon,
8500 ft., Charleston Mountains, Clark County, Nevada, August 2, 1938,
Train 2256 (N. Y. Bot. Gard.).

Known with confidence only by the type collection but strikingly marked
by its short pungent basal leaves and pungent sepals. A collection from the
nearby Sheep Range, *Alexander & Kellogg 1792,* is referred here with con-
siderable uncertainty. The specimens have the habit and leaf characters of
A. Kingii subsp. *compacta,* but the congested inflorescence more probably
aligns them with *A. congesta.*

ARENARIA CONGESTA var. **prolifera** Maguire, var. nov.

A. Burkei Howell, Fl. N. W. Am. 1: 85. 1897. Based on the original description and
the name *A. Fendleri* var. *subcongesta* S. Wats., but applied to plants of Oregon and
Washington, and not to type of var. *subcongesta.*

A. congesta var. *subcongesta* (S. Wats.), Wats. Bot. Calif. 1: 69, as to specimens
but not as to type. 1876.

A. glabrescens Piper, Contr. U. S. Nat. Herb. **11**: 261. 1906. Not *A. Fendleri* var.
glabrescens S. Wats.

Var. *congesta* similis, sed inflorescentibus proliferatis et sepalis acutis.

Similar in habit to var. *congesta* and var. *expansa,* but differing in the
open inflorescence from the former and in the longer acute sepals from the
latter.

Type locality: Junction Crab and Wilson Creeks, 1600 ft., Douglas
County, Washington, June 24, 1893, *Sandberg & Leiberg 294* (N. Y. Bot.
Gard.). Cotype: high sagebrush slopes between Coulee City and Hartline,
Grant County, Washington, June 16, 1935, *Thompson 11664* (N. Y. Bot.
Gard.).

Distribution: Sagebrush plains and slopes eastern Washington, Oregon,
and Alberta.

Representative specimens: Lake Chelan, Chelan County, Washington, June 12, 1936,
Kelly 1; Almira, Lincoln County, Washington, June 22, 1933, *Thompson 9149.*

ARENARIA CONGESTA var. SUBCONGESTA (S. Wats.) S. Wats. Bot. Calif.
1: 69. 1876.

A. Fendleri var. *subcongesta* S. Wats. Bot. King's Expl. **5**: 40, as to type. 1871.

A. Fendleri var. *glabrescens* S. Wats. Bot. King's Expl. **5**: 40, as to cited specimens
of Nevada. 1871.

A. Burkei Howell, Fl. N. W. Am. 1: 85, as to name, not specimens. 1897.

A weak variant representing transitional forms between *A. congesta* and
A. Kingii var. *glabrescens,* and/or, perhaps *A. capillaris* subsp. *americana.*
It is not impossible that the type of var. *subcongesta* may actually represent
a depauperate specimen of the latter. I am unable to make pronouncement
in this regard after careful examination of the type at Gray Herbarium, and
an isotype at the New York Botanical Garden. Since the types have been
associated historically with *A. congesta,* there is no justification for modi-
fying the nomenclature or the association.

Type locality: East Humboldt (Ruby) Mountains, Elko County, Nevada,
8000 ft., July, 1868, *S. Watson 168* (Gray Herb.).

Distribution: Plants of inhospitable habitats, Great Basin from northern Nevada and adjacent California to Millard County, Utah, and Nye County, Nevada.

Representative specimens: "California" 1875, *Lemmon 40*; Arum, Nevada, May 9, 1893, *Jones* sine no.; Ruby Hill, Nevada, July 7, 1891, *Jones* sine no.; Cisco [Millard County], Utah, May 2, 1890, *Jones* sine no.; Sherman Peak, White Pine County, Nevada, August 4, 1939, *Hitchcock & Martin 5635*; Midas, Elko County, Nevada, June 10, 1940, *Holmgren 642*; Spruce Mountain, Elko County, Nevada, July 22, 1941, *Holmgren 1506*. The last three specimens are intermediate to var. *wheelerensis*.

ARENARIA CONGESTA var. **wheelerensis** Maguire, var. nov. Caulibus 5–10 (12) cm. altis; foliis innovationibus 1.2–1.5 (2.0) cm. longis; foliis cauliniis frequenter brevioribus; sepalis (5) 5.5–6.5 mm. longis, acutis 3-nerviis; petalis 6–10 mm. longis; inflorescentibus non dense aggregatis.

Type locality: Common, open bank in spruce-aspen belt about Lake Stella, 10,500 ft., Lehman Creek Basin, Mt. Wheeler, Snake Range, White Pine County, Nevada, August 4, 1941, *Maguire 21129* (N. Y. Bot. Gard.).

Distribution: Certainly a derivative of *A. congesta* and apparently localized in the high mountain region of northeastern Nevada and possibly adjacent Utah.

Representative specimens: Snake Range, White Pine County, Nevada, *Maguire 21155, 21095, Hitchcock & Martin 5635*; Elko County, Nevada, *Holmgren 642, 1506*.

ARENARIA CONGESTA var. **simulans** Maguire, var. nov. Ab var. *wheelerensi* caulibus 10–15 cm. altis, foliis recurvatis; sepalis 1 (3)-nerviis differt.

Weakly separated morphologically from var. *wheelerensis*, but recognizable in its taller stems, usually recurved leaves, usually 1-nerved sepals, and discrete distribution.

Type locality: Plentiful in gravelly clay soil on an open slope east of Fredonyer Pass, elevation 5400 ft. Arid Transition Life Zone, Lassen County, California, June 23, 1938, *Heller 15198* (N. Y. Bot. Gard.).

Distribution: The mountain region of Modoc and Lassen Counties, California; and the Santa Rosa Range, Humboldt County, Nevada.

Representative specimens: Alturas, Modoc County, May 28, 1940, *Hitchcock 6728*; "Pot-Hole Springs" 30 mi. west Goose Lake, Modoc County, 1894, *Austin* sine no.; Madaline plains, June 1898, *Austin & Bruce* sine no.; Indian Summit, Santa Rosa Range, Humboldt County, Nevada, *Maguire & Holmgren 22504, 22514*.

7. ARENARIA FENDLERI A. Gray, Mem. Am. Acad. II. **4**: 13. 1849.

KEY TO THE VARIETIES

1. Sepals acuminate, 6–7.5 mm. long, inflorescence moderately glandular.
 2. Leaves 1–6 cm. long, ascending or recurved, inflorescence ascending.
  3. Leaves 3–6 cm. long.   *A. Fendleri* var. *Fendleri*.
  3. Leaves 1–2 (3) cm. long.   *A. Fendleri* var. *Porteri*.
 2. Leaves 6–10 cm. long, flexuous; inflorescence lax. *A. Fendleri* var. *diffusa*.
1. Sepals acute, 4–6 (7) mm. long, inflorescence densely glandular.
 2. Leaves 2–4 (6) cm. long; sepals 5–6 (7) mm. long. *A. Fendleri* var. *brevifolia*.
 2. Leaves 1–2 cm. long; sepals 4–5 (6) mm. long. . *A. Fendleri* var. *Tweedyi*.

ARENARIA FENDLERI var. **Fendleri** Maguire, var. nov.

A. Fendleri A. Gray, Mem. Am. Acad. II. 4: 13, as to type. 1849.
A. Fendleri A. Gray subsp. *genuina* Maguire, Madroño 6: 23. 1941.

Type locality: Las Vegas, San Miguel County, New Mexico, *Fendler 57* (Gray Herb.).
Distribution: The major and most extensive population; plains and mountain slopes to the alpine belt; Albany and Carbon Counties, Wyoming (and possibly adjacent Nebraska), Colorado, in the mountains south in Arizona to the Huachuca Mountains, Cochise County, in New Mexico to the Oregon Mountains, Donna Ana County, and in Texas to Jeff Davis County; probably to be found in the states of Sonora and Chihuahua, Mexico.

ARENARIA FENDLERI var. PORTERI Rydb. Bull. Torrey Club **31**: 407. 1904.

Type locality: Grays Peak, altitude 13,000 ft., August 29, 1878, *M. E. Jones 716* (N. Y. Bot. Gard.).
Distribution: The alpine form confined to mountain peaks above timber-line in Colorado, and the San Francisco Mountains, Coconino County, Arizona.

Representative specimens: Colorado: Mount Ouray, Ouray (?) County, August 20, 1901, *Baker 818, 849*; Berthoud Pass, Grand County, July 1903, *Tweedy 5533*; Silver Plume, Clear Creek County, *Shear 1600*; San Francisco Mountains, Coconino County, Arizona, August 28, 1938, *Little 1720*.

ARENARIA FENDLERI var. DIFFUSA Porter, Fl. Colo. Geol. Surv. Misc. Pub. **4**: 13. 1874.

A. laxiflora Rydb. Bull. Torrey Club 39: 316. 1912.

Type locality: Ute Pass, Colorado, *Porter*.
Distribution: Favorable sites, grassy slopes and open woodlands to 10,000 ft. Colorado and northwestern New Mexico; perhaps throughout the range of the species.

Representative specimens: Bindermeer, Jefferson County, Colorado, July 4, 1921, *Clokey 4103*; Trinidad, Las Animas County, Colorado, July 3, 1937, *Rollins 1801*; El Porvenir, San Miguel County, New Mexico, October 23, 1939, *Drouet & Richards 3324*.

ARENARIA FENDLERI var. **brevifolia** (Maguire) Maguire, comb. nov.

A. Fendleri Gray subsp. *brevifolia* Maguire, Madroño 6: 23, exclusive of var. *brevicaulis*. 1941.

Type locality: Burro Pass, La Sal Mountains, 11,300 ft., Grand County, Utah, *Maguire 17972* (Intermountain Herb.).
Distribution: Open slopes and meadows and open pine woodland, some-times occupying drier areas, Grand and San Juan Counties, Utah; Montrose, Montezuma, and La Plata Counties, Colorado; Mohave and Coconino Counties, Arizona.

Representative specimens: Horse Gulch, La Sal Mountains, Grand County, Utah, July 15, 1911, *Rydberg & Garrett 8955*; Abajo Mountains, San Juan County, Utah, July 1-2, 1930, *Goodman & Hitchcock 1408*; Kiabab Forest, Coconino County, Arizona, June 28, 1935, *Maguire 12254*.

ARENARIA FENDLERI var. **Tweedyi** (Rydb.) Maguire, comb. nov.

A. Tweedyi Rydb. Bull. Torrey Club 31: 406. 1904.
A. Fendleri brevifolia, var. *brevicaulis* Maguire, Madroño 6: 23. 1941.

Type locality: La Plata Mountains, Colorado, July 15, 1896, *Tweedy* sine no. (N. Y. Bot. Gard.).

Distribution: Alpine peaks above timberline, La Plata Mountains, La Plata County, Colorado, and the La Sal Mountains, Grand and San Juan Counties, Utah.

Representative specimens: Mt. Peal, La Sal Mountains, San Juan County, Utah, July 17, 1911, *Rydberg & Garrett 8983*; Mt. Waas, La Sal Mountains, Grand County, Utah, July 13, 1933, *Maguire et al. 17982*.

8. ARENARIA EASTWOODIAE Rydb. Bull. Torrey Club **31**: 406. 1904.

KEY TO THE VARIETIES

Stems and inflorescence glabrous.	*A. Eastwoodiae* var. *Eastwoodiae*.
Stems and inflorescence glandular.	*A. Eastwoodiae* var. *adenophora*.

ARENARIA EASTWOODIAE var. **Eastwoodiae** Maguire, var. nov.

A. Eastwoodiae, Bull. Torrey Club 31: 406, as to type. 1904.

Type locality: Grand Junction, 1892, *Alice Eastwood* sine no. (N. Y. Bot. Gard.).

Distribution: Dry stony or sandy hills, mesas, and deserts, Daggett and Uintah Counties, Utah, Mesa County, Colorado, south to northern Coconino, Navajo, and Apache Counties, Arizona, and San Juan County, New Mexico.

Representative specimens: Delta, Delta County, Colorado, July 15, 1935, *Maguire 12458*; San Raphael Swell, Emery County, Utah, June 6, 1940, *Maguire 18451*; Inscription House, Coconino County, Arizona, June 11, 1938, *Peebles & Smith 13912*; Williams, Coconino County, Arizona, June 26, 1935, *Maguire 12234*.

ARENARIA EASTWOODIAE var. ADENOPHORA Kearney and Peebles, Jour. Wash. Acad. **29**: 475. 1939.

Type locality: Tuba, Coconino County, Arizona, *Peebles & Fulton 11856* (U. S. Nat. Herb. 1634508).

Distribution: Distribution that of var. *Eastwoodiae*, but tends to be more abundant on less favorable habitats, and the only form in extreme desert areas.

Representative specimens: De Beque, Mesa County, Colorado, May 26, 1910, *Osterhout 4247*; Aztec, New Mexico, May 1899, *Baker* sine no.; Natural Bridges, San Juan County, Utah, August 4–6, 1911, *Rydberg & Garrett 9414*; Fruita, Wayne County, Utah, June 30, 1940, *Maguire 19269*.

9. ARENARIA MACRADENIA S. Wats. Proc. Am. Acad. **17**: 367. 1882.

KEY TO THE SUBSPECIES AND VARIETIES

1. Sepals 5.5–6.5 mm. long; stems woody at the base; plants of the Mohave Desert.   *A. macradenia* subsp. *macradenia*.
  2. Petals conspicuously exceeding the sepals; cauline leaves 5 or more pairs; innovations seldom produced.

3. Cauline leaves mostly 5–8 pairs, ascending, 0.8–1.2 mm. broad.
                                                         A. macradenia var. *macradenia.*
3. Cauline leaves mostly 6–12 pairs, strongly arcuate, 1.2–2.0 mm.
     broad.   .
     4. Inflorescence and sepals glabrous.   *A. macradenia* var. *arcuifolia.*
     4. Inflorescence and sepals densely glandular.   *A. macradenia* var. *Kuschei.*
   2. Petals equaling or barely exceeding the petals; cauline leaves fewer
        than 5 pairs; innovations frequently produced.   *A. macradenia* var. *Parishiorum.*
1. Sepals 4.0–5.5 mm. long; stems little or not at all woody at the base;
     plants of the Great Basin.   *A. macradenia* subsp. *Ferrisiae.*

ARENARIA MACRADENIA subsp. MACRADENIA Maguire, Bull. Torrey Club
72: 326. 1946.

   A. macradenia S. Wats. Proc. Am. Acad. 17: 367, as to type. 1882.

ARENARIA MACRADENIA subsp. MACRADENIA var. MACRADENIA Maguire,
Bull. Torrey Club **72**: 326. 1946.

   A. macradenia S. Wats. Proc. Am. Acad. 17: 367, as to type. 1882.
   A. congesta var. *macradenia* (S. Wats.) M. E. Jones, Proc. Cal. Acad. II. 5: 626. 1895.
   A. Fendleri var. *glabrescens* S. Wats. Bot. King's Expl. 5: 40, in part. 1871.

Type locality: Near Mohave River, 1876, *Palmer 41* (Gray Herb.).
Distribution: Deserts, dry slopes and foothills to 5000 ft., Inyo and Los
Angeles Counties, California; South Bern Mountains, Lower California;
Mohave Desert to Clark County, Nevada; Washington County, Utah, and
northern Mohave and Coconino Counties, Arizona.

   Representative specimens: St George, Washington County, Utah, 1877, *Palmer 53*;
Caliente, Lincoln County, Nevada, September 7, 1938, *Train 2472*; Big Pine Creek, Inyo
County, California, July 13, 1941, *Alexander & Kellogg 2545*; San Bernardino Mountains,
Bernardino County, California, May 1882, *S. B. & W. F. Parish 1329*.

ARENARIA MACRADENIA subsp. MACRADENIA var. arcuifolia Maguire, var.
nov.   Sepalis 5.5–6.5 mm. longis, ciliolatis; foliis caulinibus fere 6–12 jugis,
1.2–2.0 mm. latis, valde arcuatis.
Type locality: Dry slopes, Mint Canyon, north of San Gabriel Mountains,
May 25, 1923, *P. A. Munz 6792* (N. Y. Bot. Gard.).
Distribution: Dry slopes and foothills, San Gabriel, Liebre, and San
Antonio Mountains, Los Angeles County, California. A strongly marked
variant, recognized by the numerous, broad, arcuate cauline leaves and cili-
ate sepals.

   Representative specimens: California: Liebre Mountains, Los Angeles County, June
20–23, 1908, *Abrams & McGregor 357*; Mohave Desert, May 25, 1882, *Pringle sine no.*;
Kernville, Kern County, *Bacigalupi & Ferris 2476*.

ARENARIA MACRADENIA subsp. MACRADENIA var. Kuschei (Eastwood)
Maguire, comb. nov.

   A. Kuschei Eastw. Proc. Calif. Acad. IV. 20: 140. 1931.

Differing from var. *arcuifolia* only in the prominent glandular pubes-
cence of the inflorescence and sepals.
Apparently known only from the type: Forest Camp, Mohave Desert,
California, July 12, 1929, *August Kusche* sine no. Herbarium California
Academy of Sciences, No. 169243.

ARENARIA MACRADENIA subsp. MACRADENIA var. PARISHIORUM Robins. Proc. Am. Acad. **29**: 296. 1894.

A. congesta var. *Parishiorum* (Robins.) Robins. in Syn. Fl. N. Am. 1: 242. 1897.

This variant passes freely into var. *macradenia*.

Type locality: Common on mountains bordering on to the Mohave Desert [California], *S. B. & W. F. Parish 1330* (Gray Herb.).

Distribution: Desert ranges of southern California and southern Nevada to the Virgin Mountains, Mohave County, Arizona; "Lower California."

Representative specimens: White Mountains, Inyo County, California, May 13, 1930, *Duran 2637*; Clark County, Nevada: Valley of Fire, April 5, 1934, *Maguire et al. 4794*, and Charleston Mountains, June 6, 1936, *Clokey 7082*; Grand Wash, Mohave County, Arizona, April 26, 1941, *Maguire & Holmgren 20665*.

ARENARIA MACRADENIA subsp. FERRISIAE Abrams, Ill. Fl. Pacif. States **2**: 151. 1944.

Characterized by small flowers, less conspicuously nerved sepals, and somewhat less suffruticose base, this population is set off ecologically and to a large extent geographically from the major large-flowered desert race.

Type locality: Trail to Big Pine Lake along north fork of Big Pine Creek, elevation 9000–9500 ft., Inyo County (California), *Roxana S. Ferris 9000* (No. 230580 Dudley Herb.).

Distribution: Foothills and mountain slopes 5000–9500 ft., Inyo and Kern (according to Abrams) Counties, California, Lincoln County, Nevada, and Iron, Beaver Counties north to Utah County, Utah.

Representative specimens: Lone Pine [Inyo County], California, May 14, 1897, *Jones sine no.*; Granite Mountain Pass 15 mi. E. Milford, Beaver County, Utah, June 22, 1941, *Maguire 20998*; Mt. Nebo, Juab County, Utah, August 15, 1905, *Rydberg & Carlton 7579*.

10. ARENARIA KINGII (S. Wats.) M. E. Jones, Proc. Calif. Acad. Sci. II. **5**: 627. 1896.

This species is perhaps too broadly drawn, as was *A. congesta*. Yet if it is to be interpreted as more than a single polymorphic species, an overly great number of intermediate plants would be left without any place for assignment, or they would necessitate the erection of new specific names. But these would then rest on weak characters of indefiniteness and represent minor and loose assemblages without natural ranges. The name-bringing entity, the var *Kingii*, might easily be lifted from the major part of the population. It is always recognizable (in flower) because of its bifid petals, but otherwise it is hardly to be distinguished from the var. *glabrescens*.

KEY TO THE SUBSPECIES AND VARIETIES

1. Sepals 3.6–4.5 (5.0) mm. long; capsule 4.5–6.3 mm. long; seed 1.5–2.0 mm. long.
    2. Petals white; inflorescence open, few- to many-flowered.
        3. Stems more or less leafy; leaves acerose, slender, mostly strict or ascending, 1–2 cm. long (ssp. *Kingii*).
            4. Petals bifid, inflorescence conspicuously glandular.   *A. Kingii* var. *Kingii*.
            4. Petals entire or merely retuse, inflorescence inconspicuously glandular.   *A. Kingii* var. *glabrescens*.
        3. Stems more or less scapose; leaves basally disposed, 0.5–1.5 cm. long, broadly subulate or trigonous, ascending or divergent.

4. Leaves trigonous, ascending, feebly ciliate; inflorescence including the sepals densely glandular; plants of the high central plateaus, Utah.   *A. Kingii* subsp. *plateauensis.*

4. Leaves broadly subulate, tending to become widely divergent, prominently ciliate; inflorescence feebly glandular, sepals mostly glabrous; plants of the White Mountain and Mt. Whitney region, California.   *A. Kingii* subsp. *compacta.*

2. Petals pink, inflorescence strict, few-flowered, caudex mostly few-branched; plants of the Charleston Mountains.   *A. Kingii* subsp. *rosea.*

1. Sepals (4.5) 5.0–6.0 mm. long; capsule 5.5–7.2 mm. long; seed 2.0–2.5 mm. long; petals entire or occasionally bifid; plants of Wasatch Range, Utah, extending northward into Wyoming and Idaho.   *A. Kingii* subsp. *uintahensis.*

ARENARIA KINGII subsp. **Kingii** Maguire, subsp. nov.

Stellaria Kingii S. Wats. Bot. King's Expl. 5: 39, as to type. 1871.

ARENARIA KINGII subsp. KINGII var. **Kingii** Maguire, var. nov.

Stellaria Kingii S. Wats. Bot. King's Expl. 5: 39, as to type. 1871.

Type locality: East Humboldt [Ruby] Mountains, 9000 ft. altitude, July, 1868, *S. Watson 164* (Gray Herb.).

Distribution: Dry slopes of the Great Basin ranges, particularly in eastern Nevada and west central Utah.

Representative specimens: Deep Creek Range, Juab County, Utah, July 1943, *Maguire & Holmgren 21934, 21939*; Desert Range Experiment Station, Millard County, Utah, June 18, 1941, *Maguire 20860*; Nevada: Pioche, Lincoln County, May 2, 1939, *Train 2684*; Pequop Range, Elko County, June 2, 1934, *Maguire et al. 5899*; Snake Range, White Pine County, June 18, 1941, *Maguire 20838*; Quinn Canyon Range, Nye County, June 6, 1945, *Maguire & Holmgren 25309.*

ARENARIA KINGII subsp. KINGII var. **glabrescens** (S. Wats.) Maguire, comb. nov.

A. Fendleri var. *glabrescens* S. Wats. in Bot. King's Expl. 5: 40, as to type. 1871.

Type locality: Toiyabe Mountains, 6000 ft. [Nye County] Nevada, July, 1865, *S. Watson 167* (type, Gray Herb.; isotype, N. Y. Bot. Gard.).

Distribution: Dry hills and slopes to 8000 ft., southeastern Oregon, southwestern Idaho, south to Esmeralda County, Nevada, and Inyo County, California, and in the basin ranges to Beaver and Iron Counties, Utah.

Representative specimens: Castle Peak, Nevada County, California, July 31, 1903, *Heller 7063*; Nevada: Boundary Peak, White Mountains, Esmeralda County, June 5, 1940, *Train 3251*; Paradise Range, Nye County, *Maguire & Holmgren 25397*; Izzenhood Ranch, Lander County, *Holmgren 542, 1100*; Golliher Pasture, San Jacinto, Elko County, *Maguire 16811, 16812, Holmgren 1372* (the three Golliher Pasture collections are intermediate to *A. capillaris americana*).

ARENARIA KINGII subsp. **compacta** (Coville) Maguire, comb. nov.

A. compacta Coville, Proc. Biol. Soc. Wash. 7: 67. 1892.

The culminative reductive alpine ecotype of *A. Kingii*. The type from Whitney Meadows is an exceedingly reduced specimen. Field study on the White Mountains where the subspecies grows abundantly shows all intergradation between the type and plants that are hardly separable from the subsp. *Kingii* var. *glabrescens.*

Type locality: Timberline, divide n.w. Whitney Meadows, Sierra Nevada, Tulare County, California, August 20, 1891, *F. V. Coville 1653* (U. S. Nat. Herb.).

Distribution: Alpine meadows and ridges, Mt. Whitney Region, Tulare County, and White Mountains, Inyo and Mono Counties, California.

Representative specimens: California: Piute Pass, Great Western Divide, July 22, 1934, Inyo County, *Ferris 8885*; Wyman Creek, White Mountains, August 1, 1945, *Maguire & Holmgren 26042*; Cottonwood Creek, White Mountains, Mono County, August 8, 1945, *Maguire & Holmgren 26153*; Johnny's Corral, White Mountains, Mono County, August 6, 1945, *Maguire & Holmgren 26106* (pink-flowered form).

ARENARIA KINGII subsp. **uintahensis** (A. Nels.) Maguire, comb. nov.

A. uintahensis A. Nels. Bull. Torrey Club **26**: 7. 1899.
A. aculeata var. *utahensis* (Nels.) Peck, Madroño **6**: 133, at least as to name. 1941.

Plants with bifid petals, but otherwise identical with the larger population, occur sporadically with it, mostly in the southern part of the range. Obviously, this form with bifid petals is the correlative of the Great Basin var. *Kingii*, but evidently does not, as is the case with var. *Kingii*, form a discrete population. For this reason it is deemed inadvisable to consider the bifid individuals as constituting a formally recognizable entity.

Type locality: Cokeville, Uinta County, Wyoming, June 11, 1898, *A. Nelson 4640* (Univ. Wyo.). The specimens of the type collections are quite intermediate between *A. capillaris* and the great body of material that has been known as *A. uintahensis*. In fact, particularly in the obtuse, merely cuspidate sepals, the type more resembles the former, *A. capillaris*. However, a collection, *A. Nelson 1583*, from Leroy, Wyoming, near the type locality and designated "cotype" on the University of Wyoming sheet (but not cited in the original description), is entirely characteristic of the population "uintahensis" in having sepals "narrowly ovate, acute" as designated in the original description. It must be assumed, therefore, that Nelson's diagnosis was drawn more largely from the Leroy collection and perhaps other representative material than from the type.

Distribution: Slopes and foothills to 8000 ft., southeastern Idaho and adjacent Wyoming, south in the Wasatch Range to Sanpete and Beaver Counties, Utah.

Representative specimens: Challis, Custer County, Idaho, June 6, 1935, *Smith 9*; Bush Ranch, Sweetwater County, Wyoming, June 10, 1900, *A. Nelson 7091*; Utah: Wasatch [Range], August 1869, *S. Watson 166*; Bear River Range, Cache County, June 9, 1936, *Maguire 13670*; Vernal, Uintah County, June 16, 1937, *Rollins 1745*; Maple Canyon, Moroni, Sanpete County, June 13, 1940, *Maguire 18671*.

ARENARIA KINGII subsp. **plateauensis** Maguire, subsp. nov. Caudicibus multicipitalibus; innovationibus frequentibus; caulibus (2) 5–10 (15) cm. altis, gracilibus, dense glanduloso-pubescentibus; foliis basilaribus (innovatiorumque) (0.5) 1.0–1.5 cm. longis, triquetribus, glabris mucronatis, minute serrulatis; foliis caulinibus 1–2 jugis (rare 3–4); inflorescentibus ab medio cauline, dense glandulosis; pedicellis (3) 4–6 (10) mm. longis, tenuibus; sepalis (3.5) 4.0–5.0 (6.0) mm. longis, lanceolatis, acutis vel aliquantum obtusis, moderate glandulosis; petalis (3) 4–6 (7) mm. longis, oblongo-oblanceolatis; glandibus 0.5–1.0 mm. longis, ovalibus; capsulis 4–6 mm. longis, ovato-oblongis; seminibus 1.5–2.1 mm. longis, papillatis, nigrescentibus.

The subsp. *plateauensis* is the eastern correlative of the western subsp. *compacta,* and has frequently been there referred. It has been most often interpreted as *A. aculeata,* however, with which it has no immediate affinities.

Type locality: Abundant, open park in spruce-aspen forests, Cedar Breaks rim, 10,400 ft., Iron County, Utah, June 23, 1940, *Maguire 19024.*

Distribution: Common in pine woodlands at lower altitudes and open aspen and spruce parks and ridges at higher altitudes, from 8000 to 11,500 ft., the high plateau region of south central Utah in Iron, Beaver, Garfield, Wayne, and Kane Counties, and in the Henry Mountains, Wayne and Garfield Counties.

Representative specimens: Utah: Frequent, spruce-aspen, 9500 ft., Aquarius Plateau, ½ mi. east of pass, 8 mi. east Widtsoe, Garfield County, June 27, 1940, *Maguire 19141*; common sage park, 10,000 ft., Aquarius Plateau, 2 mi. north Cyclone Lake, Powell National Forest, Garfield County, June 29, 1944, *Maguire 19233*; common, summit Mt. Ellen, 11,500 ft., Henry Mountains, Garfield County, July 2, 1940, *Maguire 19345*; common, meadow in spruce-aspen, 9000 ft., Big John Flats, Beaver County, July 19, 1940, *Maguire 19671*; Harris Flats, Kane County, July 4, 1937, *Gierisch 453*; Panguitch Lake, Garfield County, September 6, 1894, *Jones 6007.*

ARENARIA KINGII subsp. ROSEA Maguire, Bull. Torrey Club **72**: 326. 1946.

Type locality: Hilltop, yellow pine belt, Lee Canyon, 2600 m., Charleston Mountains, Clark County, Nevada, August 4, 1935, *I. W. Clokey 5560* (N. Y. Bot. Gard. Isotypes, Clokey Herbarium, Intermountain Herbarium).

Distribution: Known only from the Charleston Mountains by the type and the following collections: *Alexander 775a, 775b; Heller 11055.*

11. ARENARIA STENOMERES Eastwood. Leafl. West. Bot. **4**: 63. 1944.

Known only from the type collection: limestone cliffs, Meadow Valley Range, Lincoln County, Nevada, May 19, 1944, *Ripley & Barneby 3476* (isotype, N. Y. Bot. Gard.).

12. ARENARIA FRANKLINII Dougl. ex Hook. Fl. Bor. Am. **1**: 101. 1831.

A. Franklinii Dougl. ex Hook. β *minor* H. & A. Bot. Beechey Voy. 326. 1838.

Type locality: Snake County at Snake Fort, *Tolmie* sine no.

This plains species constitutes a relatively uniform population. A single variation in the var. *Thompsonii* Peck (Torreya **32**: 149. 1932) with bright green herbage and short (5–6 mm.) sepals is apparently known by only the type collection, *Thompson 4769,* from Gilliam County, Oregon. Specimens of the variety have not been studied, hence it is impossible at this time to arrive at any conclusion as to the proper status of the entity.

Type collection: Abundant on barren sandy plains and undulating grounds of the Columbia, from the *"Great"* to the *"Kettle Falls," Douglas* sine no. Probably at the British Museum.

Distribution: Sand and sage plains of the Columbia and Snake River drainages, Stevens County, Washington, south to Malheur (?) County, Oregon, and Owyhee County, Idaho.

Representative specimens: Connell, Franklin County, Washington, May 1902, *Elmer 2*; Soap Lake, Grant County, Oregon, June 15, 1935, *Thompson 11623*; Reynolds Creek, Owyhee County, Idaho, July 3, 1911, *Macbride 1016.*

13. ARENARIA HOOKERI Nutt. ex Torr. & Gray, Fl. N. Am. 1: 178. 1838.

KEY TO THE VARIETIES

Leaves mostly 0.3–1.5 cm. long.   *A. Hookeri* var. *typica.*
Leaves mostly 2–4 cm. long.   *A. Hookeri* var. *pinetorum.*

ARENARIA HOOKERI var. **typica** Maguire, var. nov.

A. Hookeri Nutt. ex T. & G. Fl. N. Am. 1: 178, as to type and major portion of population. 1838.

A. Franklinii var. *Hookeri* Rydb. Bot. Surv. Neb. 3: 27. 1894.

Type locality: Rocky Mountain range, on the summits of high hills (lat. 40°), *Nuttall* (not seen).

Distribution: Plains and exposed slopes and ridges mostly east of the continental divide, southern Montana, Wyoming, adjacent Nebraska, and Colorado. One collection, *Holmgren 1573*, from northeastern Elko County, Nevada, is typical of material from the natural range but for the longer sepals (8–10 mm. in length).

Representative specimens: Wyoming: Green River, Sweetwater County, June 23, 1896, *Jones* sine no.; Cheyenne, Laramie County, July 3, 1935, *Maguire 15906*, intermediate to var. *pinetorum*; Kemmerer, Lincoln County, July 11, 1935, *Maguire & Piranian 12354*.

ARENARIA HOOKERI var. **pinetorum** (A. Nels.) Maguire, comb. nov.

A. pinetorum A. Nels. Bull. Torrey Club 26: 350. 1899.

Type locality: Laramie Peak [Albany County], Wyoming, August 7, 1895 [*Nelson*] *1595* (Univ. Wyo.).

Distribution: Open pine and spruce woodlands, southeastern Wyoming and Colorado south to El Paso County, possibly through the range of the major population.

Representative specimens: Virginia Dale, Larimer County, Colorado, *Rollins 1797*; Colorado Springs, El Paso County, Colorado, May 29, 1878, *Jones 123*.

THE NEW YORK BOTANICAL GARDEN
    NEW YORK

LEAF VARIATION IN DELPHINIUM VARIEGATUM

HARLAN LEWIS

The conformation of the leaves affords one of the more conspicuous characters for the recognition of species in the genus *Delphinium*. Therefore, a study of leaf variation is of considerable interest. *Delphinium variegatum*, like all of the California representatives of this genus, is perennial. It forms a basal rosette of leaves during the late autumn, continues a limited growth through the winter, and produces a flowering stalk in the spring, whereupon, after fruiting, it dies back to the crown.

The leaves vary in the degree of lobing from the base of the plant to the inflorescence (fig. 1). Hence, an immediate difficulty arises as to the leaf that should be selected for comparison in quantitative studies. After careful observation it was concluded that comparable leaves can be obtained by selecting the uppermost leaf of the rosette (shown by x in fig. 1). During the past four seasons this leaf has been taken as a record from all of our plants. When, as is frequently the case, a plant has a branched crown with 2 or more flowering stalks, a leaf has been taken from each rosette. These we find are almost identical (fig. 2). If the plant is maintained in the garden for two or more seasons we have a leaf record of the same plant for different years.

The question arises: are the leaves chosen from a given plant in different years actually comparable? A plant which has a long growing season before maturing forms a dense rosette, whereas one which begins growth late in the season and matures early may form but a few leaves. In the first instance, we would be selecting perhaps the tenth leaf to be formed; in the latter perhaps the fifth. Hence, our comparisons might not be valid.

Our observations show conclusively that it is the position of the leaf with reference to the elongating stem that is of greatest importance in determining the morphology of the leaf, not its numerical position. The upper rosette leaves taken from different stalks of the same plant on the same year are remarkably similar regardless of the number of leaves in the rosette.

I wish now to call attention to the year-by-year variation. In figure 3 the upper row shows a series of upper rosette leaves taken in 1944 from 6 specimens of *D. variegatum* transplanted the previous year from a colony growing in San Luis Obispo County, California. The second row shows the comparable upper rosette leaves of the same plants taken in 1945. It can be seen that the leaves of each plant vary in the same direction, increasing in the amount of dissection. In some cases the difference between the leaves of

the same plant may be greater than the maximum difference between all of the leaves in 1944 (C–CC, D–DD).
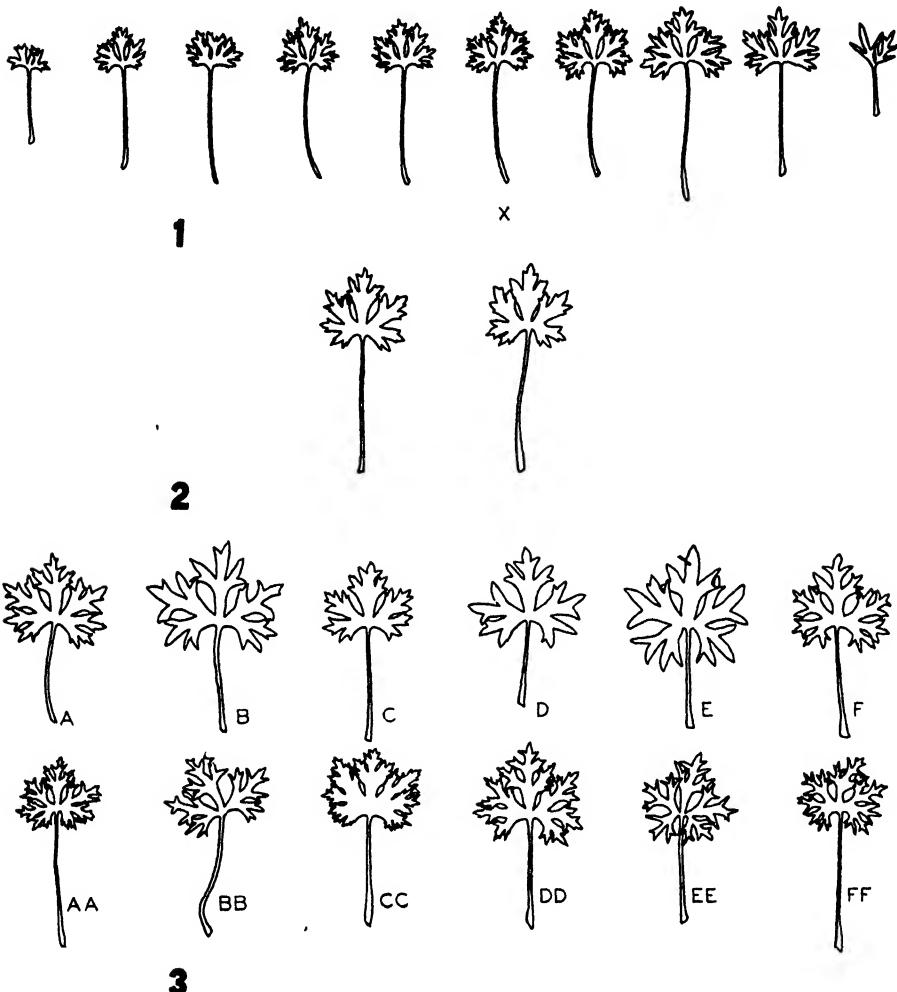
FIG. 1. The leaves produced by a plant of *D. variegatum* in one year. The lowest leaf is on the left, and the uppermost rosette leaf is indicated by x.   FIG. 2. The uppermost rosette leaf taken from two stems of the same plant in the same year.   FIG. 3. Uppermost rosette leaf of 6 plants from the same colony. Leaves taken in 1944 are indicated by single letters; corresponding leaves taken in 1945 are indicated by double letters.

All figures represent leaf tracings × ⅓.

Year-to-year variation of critical characters is a factor to be considered in studying the nature of variation of natural populations. The use of representative or mass collections in systematics for studying morphological variation has been much emphasized in recent years and has been used to con-

siderable advantage, particularly in the study of "complex" groups. In some cases the data have been suitable for the application of refined statistical techniques.

In view of the data just presented, it seems well to point out that the study of representative collections, no matter how intensively studied, may not yield as accurate an account of the variation as one perhaps would expect from the refined techniques that can be applied to such data. Certainly if the difference between comparable leaves of an individual in two successive seasons exceeds that of the leaves of all the plants in the same population in the same season, we are justified in describing that variation as no more than the range and nature of the variation for the population for the particular season chosen. The total variability of a population may, in fact, greatly exceed that indicated by a study made in one year. It may be argued that the range of variability in a population will remain constant, that is, that the same variability curve would be maintained from season to season but simply shifted in one direction or the other. Data are insufficient to demonstrate what is actually the case in *Delphinium,* but one certainly would not expect a change in any of the environmental factors to have an equal effect on every genotype; therefore, it is to be expected that the nature of the variation of a population may change from year to year, even when the same individuals are involved.

The inadequacy of the herbarium method in certain phases of systematic work has often, and justly, been pointed out. It is to be granted that there are many limitations in the use of herbarium material, one being that it is not representative of the species since there is frequently a disproportionate number of "abnormal" or exceptional individuals. However, in spite of its limitations, if a group is well represented in the herbarium, and the material is suitable for quantitative comparison, it may be possible to obtain from it more valuable information as to the nature and the range of variation of the species as a whole than if one relies only upon mass collections for his analysis, which may be much more limited with respect to space and time.

DIVISION OF BOTANY, UNIVERSITY OF CALIFORNIA

LOS ANGELES, CALIFORNIA

TROCHODENDRON, TETRACENTRON, AND THEIR MEANING IN PHYLOGENY

LEON CROIZAT

Trochodendron and *Tetracentron*, two genera of the so-called "Ranales" endemic to the Far East, have recently been investigated from the standpoint of taxonomy, anatomy, palaeobotany, and phylogeny by a group of authors (A. C. Smith, Jour. Arnold Arb. **26**: 123. 1945; Bailey & Nast, ibid. 143; Foster, ibid. 155; Nast and Bailey, ibid. 267). The reader is referred to their contributions for the data and the bibliography not mentioned in these pages.

These same genera had received prior independent attention on the part of the present writer in the course of the preparation of two works of comprehensive scope, one devoted to the generalities of angiospermous dispersal, the other to an inquiry into the phylogeny of the seed plants. Of these works the former is in final manuscript and may be published in the not too distant future; the latter, on the contrary, is now unlikely ever to see the light. Considering that *Trochodendron* and *Tetracentron* are most interesting for the phylogeny of the flower, and of the higher plants as well, it seems advisable to place on record without delay observations which might otherwise be lost and will prove of service to coming students of these subjects.

The final conclusions reached by Nast & Bailey concerning these two genera are that, although conspicuously different in several respects, *Trochodendron* and *Tetracentron* are phylogenetically close. They believe that neither has near-kinship with families which might be supposed to lie in their vicinity, Eupteleaceae, Magnoliaceae, Himantandraceae, Winteraceae, Schizandraceae, Cercidiphyllaceae, and Eucommiaceae. The conclusions of these authors, consequently, are negative, and contribute nothing to our appreciation of the lines of evolution at work in this horizon of phylogeny, and to our ultimate understanding of the so-called "Ranales."

The subject now before the writer is vast, and could justify an extensive paper. This being impossible, the limited purposes of this review must be satisfied within the compass of (a) brief notes on the ovulation, geographical distribution, and sclereids of *Trochodendron* and *Tetracentron;* (b) a study of their floral structures, reduced to bare essentials. The purpose of the writer is to leave the subject under discussion in a state fit to promote its ready use in different branches of botany and to yield the important data of which it is readily capable.

THE OVULE AND SEED

Nast & Bailey believe (op. cit. p. 274) that the vascularized, sub-chalazal projections of the ovules and seeds of the two genera appear to be unique among the Angiosperms on account of the "hair-pin" turn taken by the raphe (Nast & Bailey's "funiculus" in op. cit. pl. 4, figs. 16, 17) before entering the chalazal plate. It is pertinent to remark in this connection that "hair-pin" turns of the ovule- and seed-vasculation quite, or very nearly quite, as pronounced as those of *Trochodendron* and *Tetracentron* occur in the Thymeleaceae (*Passerina filiformis;* Guérin, Ann. Jard. Bot. Buitenz. **29** [Sér. 2, 14] : pl. 1, fig. 2. 1916. *Wikstroemia indica;* Strasburger, Flora **100**: pl. 6. fig. 1. 1910), in the Rosaceae (*Vauquelinia corymbosa;* Juel, Svensk. Vetenskaps. Akad. Handl. **58**(5): 47, fig. 81. 1918), and the Butomaceae (*Limnocharis emarginata;* Nitzschke, Cohn Beitr. **12**: 245, fig. 16. 1914).[1] A critical comparison made of these species with others in the same families (e.g., *Daphne striata, Kageneckia lanceolata, Butomus umbellatus*) or in other families (e.g., *Nepenthes;* Kühl, Bot. Centralbl. Beih. **51**(1) : 318, fig. 1. 1933) strongly suggests that these "hair-pin" figures arise, or are obliterated, even in related forms by the interplay of various secondary elements, the region of abscission of the seed, the length of the funiculus, the course of the raphe through the seed-coats, the degree of atrophy, more or less pronounced fusion of the ovular integuments. Peculiar trajects of the raphe moreover are well known in sundry families (Miers, Trans. Linn. Soc. **22**: 81, 97. 1856), and vascular "hair-pins" may seemingly be expected even in the arils or epimatia of certain conifers (*Podocarpus ferrugineus;* Sinnott, Ann. Bot. **27**: pl. 6, fig. 17. 1913, for example).

The peculiarities of the vasculation of the ovule and seed of *Trochodendron* and *Tetracentron*, consequently, cannot be stressed without regard to their homologies in families that have no phylogenetic ties with the Trochodendraceae. We are still poorly informed on the whole, but enough is already known to demonstrate that alate and exalate seeds with variable coats occur, for instance, in the Droseraceae (Wynne, Bull. Torrey Club **71**: 173, figs. 6, 9, 12. 1944) and the Nepenthaceae, the classification of which (Harms, Nat. Pflanzenf. **17b**: 756. 1936) is now based upon whether the seed is exalate, partly alate, or entirely alate. It is relevant for the purpose of this review that small, mostly alate seeds suggestive of *Trochodendron* and *Tetracentron*

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[1] A perfect "hair-pin" turn in the vasculation of the funiculus, due this time to a failure of the ovule to assume its standard position, is illustrated by Leliveld (Rec. Trav. Bot. Néerl. **32**: 569, fig. 23. 1935) in *Ulmus hollandica*. Interesting considerations, here unfortunately out of place, are suggested by a comparison of this aberration with the irregular ovulation of another ulmaceous form, *Holoptelea integrifolia* (Capoor, Bot. Centralbl. Beih. **57** A: 233. 1937). Students of the morphology and phylogeny of the ovary will find most valuable material in the Ulmaceae, Moraceae, and Urticaceae. See also mention of the tubillus-like micropyle of *Leucosyke* in a coming page.

are found in the hamamelidaceous *Liquidambar* and *Bucklandia,* whether or not it be true that these seeds have vascular "hair-pins."

The present distribution of these two genera is accounted for by A. C. Smith in much detail. They essentially belong to warm-temperate to temperate China or Japan. Bailey & Nast point out that their fossil record is extensive, dating back to the Lower Cretaceous and perhaps the Jurassic, and mention probable finds in the Rajmahal horizon of India. The two authors opine that these two genera or their immediate ancestors were once widely distributed in "Holarctica" but subsequently confined to a "relic" area in the Far East.

Trochodendron and *Tetracentron,* consequently, occupy a most critical range in dispersal, China and Japan being well known as two of the main phytogeographic centers of the world. The suggestion that they might have originated in "Holarctica" fits in nicely with the widespread belief, held among others by Berry (e.g., Geol. Soc. Am. Spec. Papers **12**: 32. 1938), that this mythical continent was the cradle of angiospermy.

A well rounded approach to the problem of dispersal requires the preliminary understanding of three main factors. (a) All life migrates along definite tracks, for no region was ever open to a lone genus or species. The writer does not agree in the least with Guppy's ultimate conclusions, but is fully satisfied that this author (*Plants, Seeds, and Currents in the West Indies and the Azores,* p. 332. 1917) is right when he acknowledges the deep impression made on him by the fact that forms so diverse as *Sphagnum* and *Carex* appear to migrate together. Likewise, the writer rejects Irmscher's generalities of interpretation as to dispersal, but does not doubt for a moment that certain dominant causes ("bewirkenden Ursachen"; Mitteil. Inst. Allg. Bot. Hamburg **8**: 364. 1929) mold the dispersal of all plants alike. (b) For the reason just given, it proves misleading to use any single group as standard to judge of tracks and dispersal, because phytogeography is of the earth at all times. (c) Isolated forms like *Trochodendron* and *Tetracentron* must be brought back to their phylogenetic center of origin before anything can be ventured on their first appearance and subsequent dispersal.

As will later be seen, the Trochodendraceae belong to the same archetypal node which released the Hamamelidaceae, Platanaceae, Nyssaceae, Cornaceae, and Saxifragaceae in a prior—when not in the very first—place. The Platanaceae are supposed to represent a classic instance of "holarctic" angiospermous origin, for *Platanus* is now wholly unknown in the southern hemisphere, being otherwise a very ancient form. Although often believed to be in the same case as the Platanaceae, the Hamamelidaceae are also, on the contrary, endemic both to Madagascar and Queensland, and, peculiarly,

enough, the genera so located, *Dicoryphe* and *Ostrearia,* appear to be related
(White in Proc. Roy. Soc. Queensl. **47**: 61. 1936), which leaves the pro-
ponents of "holarctic" origins free to speculate, but unable to produce solid
reasons for their assumptions. The Cornaceae, despite their being rife in the
Northern Hemisphere, are an "antarctic" family in the narrow sense of the
definition of Skottsberg (Plant World **18**: 129. 1915) because the genus
Griselinia occurs both in Chile and New Zealand. Peculiarly again, the aus-
tral genus *Corokia,* traditionally received under the Cornaceae following
Wangerin [Pflanzenr. **41**(4^{229}): 92. 1910], has recently been transferred to
the Saxifragaceae by Engler (Nat. Pflanzenf. **18a**: 215. 1930), which agrees
with Hallier's understanding of its true affinities (Bot. Centralbl. Beih.
39(2): 124. 1923). In Englerian classification *Corokia* falls in with
genera endemic to the Mascarenes, Australia, Lord Howe Island, New
Caledonia, and New Guinea, therefore of prevailingly "antarctic" or "old
oceanic" type of dispersal.

It is plain, consequently, that the Cornaceae and Saxifragaceae—not to
mention other families here necessarily omitted—are bound by clear phylo-
genetic ties not to "holarctica" but to the southern hemisphere. This indeed
is the case with an overwhelming majority of the angiospermous families,
and the difficulties attending the theory of "holarctic" origin for genera like
Fagus and *Nothofagus* are duly stressed by Wulff (Istor. Geogr. Rastenii:
525. 1944), who ultimately rejects Berry's conclusions (Plant World **19**: 68.
1916) as unfounded.

Allowing the matter to rest for the present, the writer emphasizes here
the existence of a major track of angiospermy which, originating somewhere
along the line New Zealand–Fiji, boldly sweeps forward through Malaysia,
ultimately to reach at least the Far East and the Himalayas.[2] This track may
suffer major disconnections also in Malaysia, but its outlines are readily dis-
cernible in the dispersal of certain "bipolar" species (e.g., *Carex Gaudi-
chaudiana, Euphrasia* sp.; Du Rietz, Acta Phytogeogr. Suec. **13**: 215. 1940),
and its inception indeed follows in the main the ancient shore so pointedly
outlined by Bryan (Jour. Proc. Soc. New So. Wales **78**: 42. 1944) among
other geophysicists. Interesting comparisons are suggested between this
ancient shore and Skottsberg's map (Proc. 6th Pacif. Sci. Congr. **4**: 706, fig.
21. 1939) showing the eastern limit of a fairly rich orchid flora.

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[2] The huge distributional gap in India, Afghanistan, and South-Eastern Persia which
as a rule either chokes off altogether, or materially interrupts this track between the
Burmese boundary and the Caucasus is suggested by the strongest evidence to depend
in part upon aridity in the ranges mentioned. It is well known, for instance, that
northern India has undergone desiccation even in historical times (Randhawa, Jour.
Bombay Nat. Hist. Soc. **45**: 558. 1945), and that Indian ranges which man saw well cov-
ered with luxuriant evergreen tropical forests of probable "Malayan" affinities about
twenty centuries ago are now sandy wastes rich in xerophytes of prevailing "African"
origin.

In conclusion, the Trochodendraceae and Tetracentraceae are by no means "holarctic," and only a lack of appreciation of their phylogenetic ties, and a general disregard for the fundamentals of phytogeography may account for hasty assumptions, or implications, in this sense. They belong to the archetypal plexus of the Hamamelidaceae, Platanaceae, Nyssaceae, Cornaceae, and Saxifragaceae, and their origin is inextricably bound up with that of all these families, and with other families in this broad affinity. They are relics and isolated offshoots of ancestors which yielded swarms of genera and species in other directions, and, anticipating the publication of a coming work, the writer ventures to assert that the origin of all these families is to be sought unmistakably in the southern hemisphere, and most likely in the present case somewhere in the existing southwestern Pacific. Suffice it to say that a typic Sino-Himalayan family like the Lardizabalaceae has two genera wholly isolated in Chile, which should readily give pause to hasty speculations about the "holarctic" origins of forms now appearing as "relics" in the Far East.

THE SCLEREIDS OF TROCHODENDRON

The skillful handling of these bodies by Foster (Jour. Arnold Arb. **26**: 155. 1945; Am. Jour. Bot. **32**: 456. 1945) and his additional work on those of *Camellia* (Bull. Torrey Club **71**: 302. 1944) readily suggest to a critical reader that our understanding of sclereids is most imperfect in anything but certain readily observable facts. Considering that sclereids—or bodies supposed to be homologous with sclereids—occur in families so diverse as the Musaceae and the Theaceae, and that the details of their organization and origin are endless—witness the comments of Duval-Jouve on the crystals of *Sagittaria* (Acad. Montpell. Mém. Sci. **8**: 170. 1872) and the notes of Vöchting on those of *Myriophyllum* (Nova Acta Acad. Leopold. Nat. Cur. **36**: 1. 1873)—we might readily agree that the subject is not yet out of the descriptive and purely speculative stage.

Foster reports (Jour. Arnold Arb. **26**: 159. 1945) that sclereids are largely confined in the petiole of *Trochodendron* to the inner, highly lacunate cortical parenchyma. The ultimate significance of this localization may still be obscure, but an interesting suggestion to assist further basic research is perhaps to be found in the circumstance that septations in the foliage and stems of very many families (Duval-Jouve, op. cit.; Briquet, Bull. Herb. Boiss. **5**: 453. 1897) seem to represent, together with various types of "nodes," a region of election for sclereids and raphids.

Duval-Jouve's most interesting work follows the breaking down of these septate structures from the petiole into the blade, and it is a matter of regret that his material did not include *Hydrocleis nymphoides* of the Butomaceae which the writer believes to be peculiarly instructive. Duval-Jouve's contri-

butions makes it at least possible to conceive of a rational approach both to the problem of the sclereids and of septate stems and petioles, which appear to be to some extent interrelated, and, once suitably approached, may contribute much which is vital to a better understanding of the phylogeny and ontogeny of the angiospermous stem and leaf.

The writer believes that the students of sclereids will be amply repaid if they critically compare these bodies with the following structures: (a) permeable cells ("Durchlasszellen") in the exodermis of the roots of the Asclepiadaceae (Francke, Planta **3**: 1. 1927), and the so-called velamen of the roots of Orchidaceae and Araceae (Goebel, Flora **115**: 1. 1922); (b) hydropotes, so-called in many aquatics (Mayr, Bot. Centralbl. Beih. **32**(1): 278. 1915); (c) lithocysts in the Moraceae and their allies, not to mention other families (Renner, Bot. Centralbl. **25**(2): 183. 1910; Ajello, Am. Jour. Bot. **28**: 589. 1941); (d) "tracheids" and similar organs in many forms, from the Monocotyledons to the Cactaceae (De Fraine, Ann. Bot. **24**: 158. 1910).[3]

The writer ventures to believe that all these bodies and organs are at bottom structures which perform or performed the same functions either in storage or in assimilation, some of them still active, others on the contrary de-activated by the oncoming of entirely new secondary structures in the stem and the leaf. The ultimate origin of the vasculation of the higher plants might perhaps be sought in a critical investigation of these mysterious organs. Like several other fields of the utmost significance for pure and applied botany, the field of research here but roughly outlined is practically untouched, which is an excuse for the writer to speak of it as he does. We face here an interplay of form and metabolism which reaches most deeply into the problem of the ultimate origins and present morphology of the modern plant, and this interplay will forever escape us if we persist in approaching it along any front but the widest. Nature carries its own requirements, which it is for us to follow, not to modify nor to deny.

Clearly, Foster is correct in stressing our present lack of knowledge, and in questioning the very validity of standard definitions in current textbooks. Nothing can be concluded, of course from the presence of sclereids in *Trochodendron*, for there are too many families altogether unrelated to the Trochodendraceae which exhibit these organs; but their localization within

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[3] *Salicornia* would seem to be peculiarly instructive in this connection. According to De Fraine (Jour. Linn. Soc. **41**: 331–334. 1913) "stereides" and "spiral cells" are in some measure interchangeable among different species under this genus. In *S. glauca*, for instance, there are "abundant stereides" around the vascular cylinder of the stem just below the nodal region, and also in the free leaf-tip, where "transfusion tissue" is located in the allied *Halocnemum strobilaceum*. Only stereides occur in *S. glauca*, but in other species, as *S. pusilla, S. ramosissima* etc., spiral cells exclusively appear. Both stereides and spiral cells, finally, are present in *S. fruticosa* and a few other species.

the spongy parenchyma of the petiole of *Trochodendron* is at least suggestive in the directions here but superficially indicated.

THE FLOWERS OF TROCHODENDRON, TETRACENTRON, AND THEIR ALLIES

Judged solely from the standpoint of the flower (figs. 1, 2) *Trochodendron* and *Tetracentron* appear to be unrelated. Sharp morphological differences separate these structures at a glance, nor is any suggestion readily forthcoming on how one has evolved into the other. Unmistakable evidence of ultimate kinship, however, is at hand from a sum of characters of positive and negative order, which proclaims that these two genera are united, after all, by common bonds of consanguineity.

The problem which these two genera offer is one which a systematist is frequently called upon to elucidate and to resolve. This problem invites in the first place a consideration of the flower in general, for its prime requirement is to ascertain the nature of the evolutionary trends, which, starting from a common ancestral form, ultimately cause different structures to come into being by gradual or abrupt modifications of their organs. The flower is one of the first standards of botanical investigations, if not the very first, and its pre-eminence endures throughout.

Common sense dictates that the flowers of *Trochodendron* and *Tetracentron* should be compared with the flowers of other forms which lie at the same horizon of phylogeny, in order to identify with reasonable accuracy the level of evolution which belongs to the two genera. Once this level is detected further constructive study becomes easy. It matters little indeed whether or not *Trochodendron* or *Tetracentron* are to be treated as members of this or that family or order, but it matters a great deal that they be properly placed and understood against the background of the evolutionary trends which they share.

Hallier pointed out (Bot. Centralbl. Beih. **14:** 247. 1903) that the Trochodendraceae and the Magnoliaceae are connected with the so-called Amentiferae through the Hamamelidaceae, and Solereder maintained (Ber. Deuts. Bot. Ges. **17:** 387. 1899) that the Trochodendraceae are related with the Hamamelidaceae through genera like *Cercidiphyllum* and *Eucommia*. Generally speaking, Hallier's work proves most confusing to all of its readers who are not already quite familiar with the groups to which this author summarily refers in the pages of his over-meaty treatments. Like Baillon's classification, Hallier's conclusions are not popular writing, and for this very reason, whether right or wrong, they seldom receive their dues. Solereder, of course, is best known as a wood anatomist, and is sought after as a purveyor of facts rather than as a full-fledged systematist.

Whatever be the ultimate merit of their contributions in certain details, both Hallier and Solereder are on record as having detected a measure of

kinship between the Trochodendraceae and the Hamamelidaceae. Horne believes (Trans. Linn. Soc. II. **8**: 239. 1914) that the Hamamelidaceae—which he calls "fairly homogeneous"—can be brought back for their origins to a "pro-Saxifragean plexus." This belief agrees in substance with Baillon's (Hist. Pl. **3**: 325. 1872) inclusions of the Hamamelidaceae, Platanaceae, and the like, within the Saxifragaceae. None of the literature here cited is reported by Nast & Bailey, who might be familiar with it, but seem to have accorded it no importance.[4] This rejection does not seem to be justified, for Hallier, Solereder, Horne, and Baillon had some knowledge of plant life, and Dutailly's obituary of Baillon (Bull. Soc. Linn. Paris **2**(No. 153): 1209. 1896) pointedly emphasizes that this master botanist, a man of wide culture and a lover of fine arts to boots, "fut de tous les botanistes qui ont vécu jusqu' à ce jour, celui qui tint dans ses mains le plus de plantes."

As a preliminary toward dealing with *Trochodendron* and *Tetracentron*, we may review somewhat briefly certain forms suggested as relevant to our purposes by the authors mentioned, such as *Liquidambar*, *Platanus*, *Davidia*, and the like. The reader is soon to perceive that the time so spent is quite as usefully employed as that devoted to peering through a microscope, if this peering is done without having effected a prior broad survey of the field of investigation that is ultimately to come under the binocular.

Liquidambar is variously treated as a genus of the Hamamelidaceae or Altingiaceae, which is here irrelevant, for, treated as any one may please, *Liquidambar* certainly belongs under the hamamelioid wings. Its flowers are coacervated within heads or balls which appear as the buds first unfold, the ♂ heads being usually borne above the ♀ within elongate rameal inflorescences.

These heads are developed either to maleness or femaleness, but the separation of the sexes, though fairly sharp on the whole, is seldom absolute. The female head contains a variable number of flowers each consisting of two carpels (rarely one) surrounded by a characteristic whorl or rings of scales which repeat their origin from the mass of the head below, or torus. The flowers are tightly crowded, so that the scales belonging to any one are hemmed in by those of the others. Along the commissural lines so formed appear glandular bodies with a tumid papillate head, very prominent at the

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[4] The works of several authors, including those of Hallier and Solereder here cited, are indeed mentioned by A. C. Smith in his "Taxonomic review of *Trochodendron* and *Tetracentron*" (op. cit. 127) as having some historical significance. Of Hallier's phylogeny in regard to these genera, *Eucommia*, *Cercidiphyllum*, and *Euptelea*, Smith curtly states, "It is an opinion which has found no supporters and which is scarcely substantiated by the facts." It is a matter of regret that this dismissal is unsupported by a critical comment to demonstrate how the facts scarcely substantiate fruitful comparison of *Trochodendron* and *Tetracentron* with the Hamamelidaceae; the reader of these notes is now offered the opportunity to settle this for himself. Smith's assumption is ready proof that Hallier is all too often misunderstood.

time of anthesis, which are shown to be abortive stamens, or staminodia, by
the fact that occasionally some of them shed pollen from well formed thecae.
Whether staminodes of the same kind sometimes arise also between the em-
bossed heads of the scale remains to be ascertained; at any rate, the scales,
at first inconspicuous and hidden beneath the towering staminodes, develop
as the head mature, and ultimate become manifest and woody. The ♂ head
tends to elongate, unlike the ♀ which retains a sphaeric shape throughout.
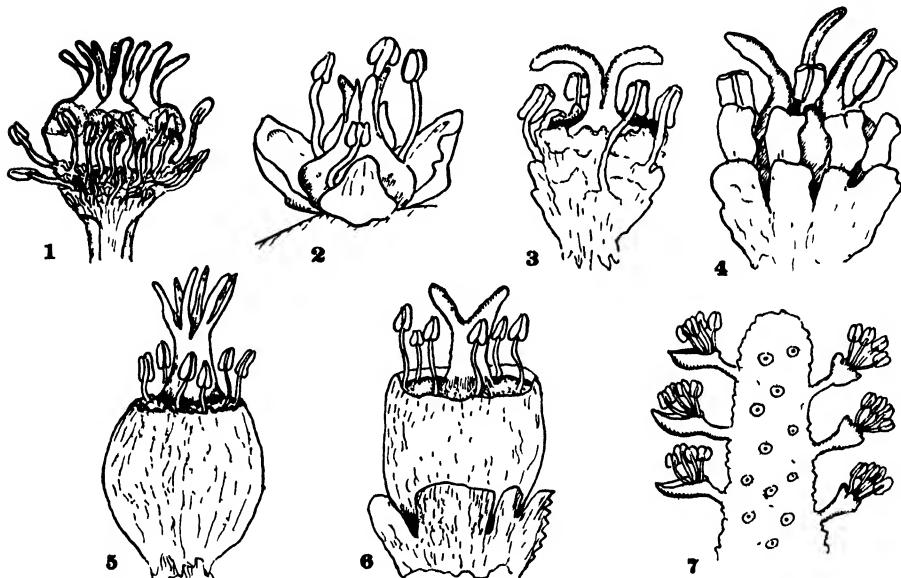
FIG. 1. A flower of *Trochodendron*. The glands on the back of the carpels are stippled.
The numerous stamens are borne upon much reduced scales, and the whole is subtended
by a pair of bracts.   FIG. 2. A flower of *Tetracentron*.   FIG. 3. A single flower of *Liquidambar* surrounded by scales bearing staminodes.   FIG. 4. A single flower of *Platanus*
(reduced to three carpels, each bearing a stamen dorsally). Staminodes are below the
stamens, and the whole is surrounded by toral emergences or scales.   FIG. 5. A flower of
Davidia, the stamens set amid minute scales.   FIG. 6. A flower of *Camptotheca*. The
stamens are set around a tissue of glandular appearance. The outer "perianth" is surrounded by toral emergences or scales, usually inclosing more than one flower.   FIG. 7. A
diagram to show derivations from the purely ♂ inflorescence of *Davidia* (The florets facing the reader are supposed to be.cut off). At the right actinomorphic perianths (quercoid
type); on the left zygomorphic or scale like perianths induced by dorsiventrality (myricoid type). (All figures diagrammatic.)

It contains very numerous stamens which appear to be set around wholly
abortive carpels. While the ♀ head normally carries staminodes, even stamens, the ♂ inflorescence is seldom anything but unisexual.

A question that still requires investigation is in the ultimate origin of
the staminodes or stamens of the ♀ head, whether they arise from toral tissues between the scales of adjacent flowers, or actually belong to the dorsal

(abaxial) surface of these scales. The matter is minor, for the scales themselves are from the body of the head, or torus, which is to say that the staminodes either belong to the scales that immediately surround the carpels, or to a row of subsidiary, undeveloped scales abaxially located from these. In this knowledge, and leaving details open for the present, a single flower from the ♀ head of Liquidambar may be figured in a manner (fig. 3) which answers both possibilities, and is basically acceptable for the purposes of this brief review.

The monotypic Platanaceae are notoriously allied with the Hamamelidaceae. The inflorescences of *Platanus* have been investigated by Brouwer (Rec. Trav. Bot. Néerl. **21**: 369. 1924) with startling results. While the flowers of this genus have constantly been accepted as actinomorphic, Brouwer shows that if there is any part of the balls of *Platanus* which may be called a "flower" this is a group constituted by a single stamen with a single carpel. These two sexual bodies are intimately connate and both receive derivations from the same ultimate vasculation. The stamen is much larger than the carpel, so large as a matter of fact that under certain conditions it appears to carry a basal ovule. In some cases the stamen is abortive, and the carpel then carries upon its back a staminode of more or less pronounced glandular nature.

A variable number of these sexual units, each consisting of a stamen, or staminode, and a carpel, are grouped in a pseudocyclic manner at definite regions of the head, which creates the illusion of actinomorphy. Certain heads are entirely ♂, others altogether ♀, many stamens being usually suppressed or reduced to staminodial condition. Heads evolving to maleness are usually located higher than those of the ♀ sex.

The heads or balls of *Platanus* are commonly believed to be connected by branch-like peduncles, but Brouwer shows that this, too, is an illusion, for each system or group of heads actually forms a single inflorescence, which consists of a central head surrounded by other heads arranged more or less in a spiral manner. Under each pseudo-flower in the head there is a slight projection from the torus which carries the stamen-carpel units in more or less regular whorls, and this projection is surrounded by an evascular scale, which Brouwer describes as a "proliferation of the ball" but illustrates most unfortunately in a manner which is not altogether clear. The purely ♂ or ♀ flower arises by abortion of one sex.

A false flower of *Platanus*, consequently, may be figured in the light of Brouwer's data in the manner here shown (fig. 4), which agrees with its conventional representations in the literature. The inflorescence itself, of course, is a peculiar thelome or phyllome (whichever be the term a reader may prefer, for the writer is not interested in standard definitions, and even less in coining and using new terms), reminiscent in some measure at least of the inflorescence of the moraceous (or urticaceous) genus *Cecropia*.

Davidia, a peculiar monotype credited either to the Cornaceae or Nyssaceae, has been carefully studied by Horne (Trans. Linn. Soc. II. **7**: 303. 1909) both as to general and floral anatomy. It is a matter of regret that nothing can be said here of the anatomy of the seedling, which is of the utmost interest for various fields of interpretive botany. As for the inflorescence, this consists of a globular head, subtended by two conspicuous bracts, which earned for this genus its common name "dove tree." This head contains very numerous ♂ flowers, and a single zygomorphic ♀ flower, which might properly be called bisexual on account of the presence of a certain number of stamens, as it will soon be stated.

The stamens of the ♂ part of the head are very numerous, and are clustered at first in groups within depressions of the torus, or foveolae. Later these foveolae are lifted by toral growth so that they form minute cups with short pedicels. The prevailingly ♀ flower, at first hidden within the mass of the purely ♂ part of the head, next emerges from a low pedicel. This flower has no "perianth," but carries about 15–26 stamens at the base of the compound style, which stamens appear between bodies described as "exceedingly minute excrescences" by Horne. The ovary is of 6–9 carpels basally fused, and the single ovule in each carpel is fed by vasculation streaming in from the adjacent septa. The stamens of the prevailingly ♀ flower are serially arranged in an oppositilocular manner.

Leaving out of reckoning the purely ♂ part of the inflorescence, which is amply illustrated by Horne and other authors, the bisexual flower of *Davidia* may be figured in the manner here shown (fig. 5).

Camptotheca is another genus of the Nyssaceae or Cornaceae close to *Davidia.* The flowers are sometimes ♂ by abortion, so far as the writer has been able to ascertain, but do not differ in essentials from the seemingly bisexual ones (Hu, Ic. Pl. Sin. **1**: pl. 41. 1927), one of which is represented here (fig. 6). So far as is known to the writer, these flowers are borne in pairs upon a head or ball, and are surrounded at the base by a variable number of scales emerging from the head as toral outgrowths.

Strictures of space make it impossible to enter into details in this review, and the reader is referred to the works cited for much additional information. Beyond question, other data of considerable significance will repay the efforts of acute investigators who in due course turn their attention upon the genera and forms here dealt with, critically investigating them on a broad comparative front. As the evidence reads which is now available, however, several conclusions are indicated as necessary.

The first and most important conclusion is that these genera, understood as a whole, lie at that cross-road of evolution where amentiferous forms easily become perfect-flowered. To illustrate the point, let us take for example the

inflorescence of *Davidia* and break it into its two essential parts, ♂ and ♀. The ♂ part of this inflorescence becomes a head or ament upon which the individual flowers, borne actinomorphically or zygomorphically, are of a pattern which suggest (fig. 7) either the Myricaceae or the Fagaceae. The ♀ flower, shorn of its stamens, would be close indeed to that of *Castanea*, and, characteristically (Tognini, Atti Ist. Bot. Pavia, Nuova Ser. **3**: 1. 1894), *Castanea*, too, often carries stamens disposed precisely like those of *Davidia*. It is also clear that a prevailingly ♀ flower of *Liquidambar*, shorn of its staminodes, has the basic outline of a ♀ flower of *Juglans* (fig. 8), which consists of paired carpels, inclosed within scales. Characteristically once again, the scales of *Juglans* and its allies (C. De Candolle, Ann. Sci. Nat. Sér. 4 **18**: 5. 1862) may evolve in maleness, yielding bisexual flowers. All this is of course less than surprising, for, as Brouwer indicates, the basic constituent elements of all these flowers are emergences or scales which readily evolve in the direction of maleness or femaleness, or become abortive as staminodes and perianth-appendages of potentially undefined status. A glance at Eichler's classic diagrams (Blüthendiagr. **2**: 36. 1878) reveals that the scales evolving in full femaleness, or carpels, may take different positions in regard to the subtending bracts and axes in various juglandaceous genera. As a matter of fact, aberrant fruits of these genera are known to all keen students of plant-life, in which sterile loculation shows up, sometimes in very regular patterns, leading these students to infer rightly that the fruit of the walnut was originally a head of congested carpels within an undetermined number of scales.

In short, the vexed question, how and when the amentiferous plants become possessors of actinomorphic bisexual flowers is easily and readily answered along the lines just suggested. The sexuality of the so-called Amentiferae is definite as a rule in one direction only, though stamens or staminodes are by no means of rare occurrence in *Castanea*, and not uncommon in the ♀ flowers of other genera in that group. *Liquidambar, Platanus, Davidia, Camptotheca* are in active evolution away from the amentiferous stage, still retaining the head- or ament-like inflorescences and an ambiguous sexual expression, which leads on occasion to purely ♂ or ♀ heads or flowers.

The second conclusion is that, broadly speaking, the passage between flower and inflorescence, despite endless variants *of detail*, is at bottom anything but speculative and fraught with the difficulties which the literature contains. A fruit of the hamamelidaceous *Rhodoleia Championii* (Harms, Nat. Pflanzenf. **18a**: 335. 1930) suggests to the unwary a structure wholly homologous to a head of *Liquidambar*. However, if the flower itself be dissected, it will readily be learned that this flower consists of a variable number of bicarpellate ovaries (usually 5–6, occasionally 1, as shown by Harms)

fitted abaxially (fig. 9) with stamens[5] and conspicuous petaloid staminodes. The sepals are "concrescent," according to Harms, but could as well be described as toral emergences, for they belong not to individual ovaries but to the whole of the flower, which is in reality exactly intermediate between a true flower and an inflorescence. Naturally, if this peculiar structure be loosened up, as it were (fig. 10), and each ovary surrounded by stamens and petals (staminodes) throughout, a string of perfect bisexual flowers would appear to be compared with those of the Hamamelidaceae having absolutely actinomorphic bisexual perianths, *Fothergilla* or *Hamamelis* for example.

The third and last conclusion suggested here by these all too short considerations is that morphologists and anatomists who are not familiar with plant life in general often argue nugatory issues, mistaking their own definitions for something that nature wills but in reality abhors. Whether the ovule be "axile" or "borne upon the margins of the carpellary leaf," for example, is an issue which no one may take seriously who knows that within the closed chamber of an ovary less than 5 millimeters broad, for instance, vascular connections may take circuits quite as variable as those appearing in the ovule itself, and that "axile" and the "foliar" at this point are figments. Indeed, some ovules may be "axile," as those of the Araceae (Campbell, Ann. Bot. **14**: 20. 1900), but it is probable, as this author admits, that even in this family forms can be detected in which the ovules are borne on the "carpellary leaf." The issue is here with variants of which many are possible and probable, and variants of the kind are meaningless in the face of the basic realities of flower-structure and flower-phylogeny. Ink has flown in torrents to debate the origin of the stigma as something involved and mysterious, but in the Urticaceae, for instance, we find the seed coat (*Leucosyke capitellata*, fig. 11; Fagerlind, Svensk Vetenskaps. Akad. Handl. **21**(4): 11, fig. 2, b. 1944) produced into a so-called tubillus of *Ephedra* flavor. Contrariwise, the origin of the stigma need not worry us at all in a case like that

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[5] The descriptions of nearly all the forms mentioned in this review are indifferent or poor in the literature now current. This is understandable, considering that the flowers of these genera cannot be interpreted in the light of the inadequate concepts of floral morphology commonly accepted today, witness the basic misinterpretation of the vasculation of the flower of *Trochodendron* by Nast & Bailey later documented in these pages. Harms' treatment of the Hamamelidaceae is unusually good from the descriptive standpoint, but a slight inconsistency appears in his handling of *Rhodoleia*. Harms' description correctly reports that staminodes (petals) and stamens fully evolve only around the outer (abaxial) side of the dimerous ovaries, and are rudimentary or altogether missing elsewhere, but the figure that illustrates the genus (op. cit. p. 335, fig. 175 C) appears to belie the description. It seems probable that this figure does not correspond to the truth, as the writer learned by actual dissections which confirmed Harms's diagnosis. It should be added, however, that these forms are so variable in details that departures from the norm are anything but impossible, and only abundant material may help to settle every detail with finality. Cleancut concepts, consequently, count far more in interpreting these forms than preoccupations with the fact that stamens fail on occasion to become petaloid, or that rudimentary staminodes turn up where none at all are supposed to occur.

of *Coleanthus subtilis* (Schnarf, Vergl. Embryol. Angiosp. 267. 1931), for a pollen grain falling anywhere on the lips of a carpel of this kind (fig. 12) will readily find its way to the micropyle by simply sending its tube downward. Since a leaf often ends in a secretory hydathode, it is not surprising
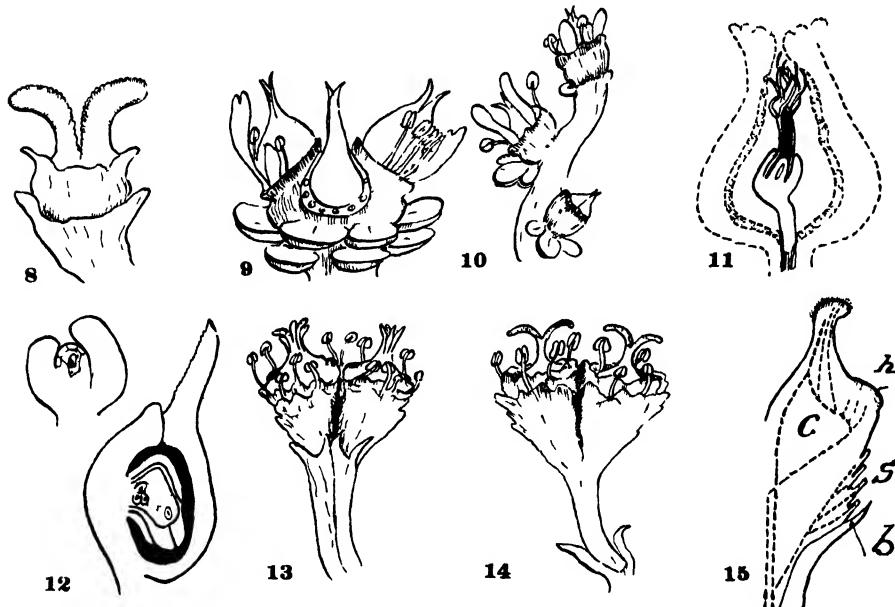
FIG. 8. A ♀ flower of *Juglans*. FIG. 9. A flower of *Rhodoleia Championii* (reduced to three 2-carpellate ovaries). The ovaries are set within a head, and embraced at base by its emergences which give origin to stamens and petals (staminodes) in an abaxial (outer) position. The "sepals" (lowermost tiers) as total emergences common to the whole head, which they surround. FIG. 10. Fully *actinomorphic* hamamelidaceous flowers (type of *Hamamelis* or *Fothergilla*) derived from an elongation of the head of *Rhodoleia Championii*. FIG. 11. Young ovary of *Leucosyke capitellata* (adapted from Fagerlind, as cited in text), showing the tubillus-like extension of the inner ovular tegument. The region stippled is the inner layer of the carpels. Were this region only extant, this ovary would be structurally and functionally quite akin that of the Ephedraceae. FIG. 12. Upper left: Very young ovary of *Coleanthus subtilis* (Gramineae); lower right: The same fully developed (adapted from Schnarf, as cited in text). FIG. 13. Diagram to show two flowers of *Trochodendron* brought together within a single head. FIG. 14. Diagram to show two flowers of *Liquidambar* arranged like those of *Trochodendron* in figure 13. FIG. 15. Vasculation of the flower of *Trochodendron* (adapted from Nast & Bailey, as cited in text). *c*, body of the carpel; *h*, the dorsal gland with convergent suggestive vasculation; *s*, the staminal region and its ridges; *b*, the subtending bract. (All figures diagrammatic.)

that a carpel may end with a similar structure, and that the pollen should find this structure, usually rich in sugar and starch, convenient.

A flower of *Trochodendron* (fig. 1) hardly differs from one of *Liquidambar* (fig. 3) when both are critically examined, for the structural essentials are the same in both, despite the greater number of carpels in the former.

Supposing that two flowers of the former are bunched together (fig. 13) it is not easy indeed to find how they differ from two flowers of the latter (fig. 14) arranged in the same manner. Considering that the basic unit in all these flowers is the pair of scales evolved in maleness and femaleness, or sterilized as staminodes, it is less than surprising that the carpel of *Trochodendron* should bear upon its back a glandular body, for an homologous development is in *Platanus,* and the stamens are oppositilocular in *Davidia*. Nast & Bailey find (Jour. Arnold Arb. **26**: 269. 1945) that the stamens of *Trochodendron* are "borne individually upon cushions that tend to be linearly arranged on more or less embossed and decurrent ridges on the dorsal surface of the carpels," but it is evident that they take a mistaken view of what constitutes the "dorsal surface of the carpels." Their own drawing of the vasculation of the flower of *Trochodendron* reveals that the vasculation abutting on the staminal region definitely originates at a lower level than the supply of the carpels (fig. 15), and that the stamens on "cushions . . . on more or less embossed and decurrent ridges" do not belong to the "dorsal surface of the carpels" but to a much reduced system of scales, homologous with those of *Liquidambar* and *Davidia*. The bracts under the flower of *Trochodendron* which Nast & Bailey decline to interpret on account of the lack of vasculation, though suggesting that they might be the equivalent of a calyx, may well be viewed as bracts or sepals, for the conceptual limits between bracts, sepals, and toral emergences lose their value at this horizon of morphology and phylogeny.

Considering that *Liquidambar* coexists under the Hamamelidaceae with *Hamamelis* and *Rhodoleia,* it is not difficult to understand how *Trochodendron* and *Tetracentron* are consanguineous despite the differences in their flowers. The same potential of evolution which yields the flower of *Rhodoleia* from that of *Liquidambar* is fit to yield that of *Tetracentron* from that of *Trochodendron,* for all these genera and flowers lie at one and the same evolutionary level. Space forbids to enter into minute details, but reference to the notes given for *Rhodoleia* may guide the reader to form his own conclutions about these details.

In short, *Trochodendron* and *Tetracentron* are isolated offshoots of a truly colossal phylogenetic plexus which is responsible for the evolution of the Hamamelidaceae, Cornaceae, and Saxifragaceae as the main families. The "Ranales" are a pure figment, for, narrowly understood, they must be restricted to forms having numerous stamens in a cyclic arrangement and heads of carpels; that is to say, the Ranunculaceae and Magnoliaceae in the main. Unduly enlarged to include the Trochodendraceae, Tetracentraceae, Cercidiphyllaceae, and the like, which hark back to a line of evolution entirely at variance with that of the Ranunculaceae and Magnoliaceae, the "Ranales" are meaningless, because *Platanus* and *Davidia* then become

"Ranalian," quite as much as the humble buttercup and the showy peony are.

DISCUSSION

The trend strongly active in botany against that branch of the science which is hazily referred to as "taxonomy" is fraught with mischief. The study of botany has been, is, and forever will be bound up with a well-diversified knowledge of plant life, and specialized investigation which neglects broad preliminary surveys of its chosen field, on the specious ground that surveys of the kind may be dispensed with, merely fills the literature with data which only those may really interpret who are able to effect such surveys in the first place. The herbarium and the botanical garden still stand as the first instruments of competent and well rounded botanical teaching, and students taught at the very first only that which is *inside* the plant without being at the same time educated to an appreciation of what is *outside* (that is, form and its corollary, potential of evolution) are fated to ultimately lose their bearings among the incredible wealth of evolved forms which enlivens plants in all their aspects.

The unfavorable results of a lack of broad surveys preliminary to specialized investigation detected in this review are apparent throughout botany. When Wetmore suggests (Torreya **43**: 21. 1943) if only by implication, that "certain seeming cauline bundles" in the cactus *Trichocereus Spachianus* may have a connection with the structures at the growing point of *Hippuris,* he fails to notice that the "cauline bundles" of *Trichocereus* (Boke, Am. Jour. Bot. **28**: 656. 1941) are unknown in another cactus, *Opuntia cylindrica,* also studied by Boke in the same paper. The reason for this difference is indeed simple. *Trichocereus* is wholly leafless, while *Opuntia* is not so; therefore the vasculation which should normally originate from, or abut on, the leaf of *Trichocereus* remains "cauline" of necessity. In certain succulents at least (e.g., *Salicornia;* De Fraine, Jour. Linn. Soc. **41**: 317. 1913) the whole of the outer cortex is foliar in origin, so that vasculation in the cortex of a leafless succulent like *Trichocereus* does not suggest a profitable field of comparison with the "cauline" vasculation of the growing tip of such other form as *Hippuris,* this genus lying wholly outside the morphological and phylogenetic field of *Trichocereus.* A proper survey of the matter indicates that *Hippuris,* a "microphyllous" genus, should be critically compared in the first place with its next of kin in the taxonomic and systematic sense. *Gunnera* takes first place among the genera to be investigated in relation to *Hippuris* and its typical growing point (Louis, Cellule **44**: 87. 1935), for it is closely allied with *Hippuris,* as a glance at Engler & Harm's *Syllabus* immediately reveals. Unlike *Hippuris,* however, *Gunnera* is a typical "macrophyllous" form. Its stem is quite as deeply dissected by the foliar traces as that of the Pteridophytes (Merker, Flora **72**: 211. 1889), but the ancient

"microphyllous" foliage of the ancestral form presumably common to it and *Hippuris* survives in the shape of peculiar bracts well illustrated by Skottsberg (Svensk. Bot. Tidskr. **22**: 392. 1928). Here, then, are subjects of investigation which handled under competent guidance not only promise results of the greatest importance as to the nature of the growing point of the Angiosperms, but herald most fruitful comparisons with the foliar differences among seedless plants, which Jeffrey wished to be fundamental (Anat. Woody Pl. 244. 1917) in obedience to dicta that answer only an anatomist's dreams. To compare *Hippuris* and *Trichocereus* on the strength that presumably "cauline bundles" belong, or might belong, to both genera leads simply nowhere.

SUMMARY

Implementing and correcting previous work written by various authors on two supposed "Ranalian" genera, *Trochodendron* and *Tetracentron*, the writer shows that these two genera are ultimately allied with the broad hamamelidoid-saxifragoid plexus as isolated forms. He briefly discusses their dispersal, ovulation, and floral peculiarities, and the sclereid problem. Incidental notes are offered on the phylogeny of the flower in general, and it is concluded that specialized investigation falls wide of its goals when it is dissociated from broad surveys preliminary to study.

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NOTEWORTHY PLANTS OF SOUTH AMERICA—III.
SPECIMENS OF MAURIA[1]

FRED A. BARKLEY

Among recent specimens studied were a series of eight collections of *Mauria*, representing for the most part species poorly represented in American herbaria or undescribed species.

Four of the specimens seem referable to described species: *J. Cuatrecasas 11370* (27 Dec. 1940, márgines del Río Mocoa, 570 m. alt., Comisaría del Putumayo, Colombia, in Herb. U. S. Nat.) and *J. Cuatrecasas, R. E. Schultes & E. Smith 12341* (17 Oct. 1941, 1300 m. alt., región del Sarare, Departamento Norte de Santander, Cordillera Oriental, Colombia, in Herb. U. S. Nat.), are *Mauria suaveolens* R & P. Agreeing very closely with the original description and photograph of the type of *Mauria birringo* Tulasne is the specimen *A. Dugand & R. Jaramillo 2952* (1500 m. alt., 4 May 1941, arribe de "El Colegio," vertiente occidental de la Cordillera Oriental, Departamento de Cundinamarca, Columbia, in Herb. U. S. Nat.). *Delgado 13* [Mar. 1937, "arbol de 10 a 12 m de altura," silvas del Avila, Venezuela, in Herb. C. N. H. (Field) M.], is *Mauria heterophylla* H.B.K.

Four of the specimens appear to represent undescribed species.

Mauria Killipii[2] Barkley. sp. nov.   Arbor 3–4 m. alta; ramis paucis, lenticellis prominentibus, striatis, dense ferrugineo-tomentosis vel cinarescentibus glabris; foliis pinnate 3-foliolatis, circa 10 cm. longis; petiolis crassis, circa 20 mm. longis, pilosis; foliolis lateralibus 2–4 mm. petiolulatis, foliolis terminalibus 6–12 mm. petiolulatis, petiolulis dense pilosis; foliolis membranaceis, utrinque prominente reticulata-nerviis, supra glabris, infra ferrugineo-tomentosis ad nervum, ovatis vel late lanceolatis, ad apicem acutis vel subacutis, ad basem late cuneatis, marginibus integris; paniculis axillaribus terminalibusque folio aequilongis; pedunculis sparse pilosis; bracteis deltoidis, sparse pilosis, ciliatis, subacutis, 1–1.5 mm. longis, subpersistentibus; calycibus circa 1.5 mm. diametro, lobis 5, late deltoideis vel late rotundo-deltoideis, sparse pilosulis, ciliatis, circa 0.3 mm. longis; petalis 5, ovatis, glabris, circa 1.3 mm. longis; staminibus 10, circa 0.45 mm. longis, antheris rotundis, flavis, circa 0.3 mm. diametro.

Tree 3 to 4 m. high, few branched and with straight trunk; branchlets yellowish brown with prominent lenticels, striate, at first ferrugineously pilose but soon glabrate; leaves pinnately 3-foliolate, about 10 cm. long; petiole coarse, about 20 mm. long, pilose; lateral leaflets short-petiolulate

[1] Publication is assisted by a contribution from the author.
[2] For Ellsworth P. Killip.

with petiolules about 2 to 4 mm. long, terminal leaflet petiolulate with peti-
olules about 6 to 12 mm. long, petiolules densely pilose; leaflets thinly mem-
branaceous, with a reticulum of veinules showing prominently, glabrous
above, the veins and venules below ferrugineously pilose, ovate (or rarely
the terminal only broadly lanceolate), acute or subacute at apex, broadly
cuneate at base, margin entire; inflorescences terminal thyrsus and lateral
panicles; peduncle, rachis and branches of inflorescence sparsely pilose;
bracts deltoid, sparsely pilose, ciliate, subacute, 1 to 1.5 mm. long, subper-
sistent; calyx about 1.5 mm. in diameter, calyx lobes 5, broadly deltoid to
broadly rotund-deltoid, sparsely short appressed pilose, ciliate, about 0.3 mm.
long; petals 5, ovate, greenish, glabrous, about 1.3 mm. long; stamens 10;
filaments filiform, about 0.3 mm. long; anthers rotund, yellow, about 0.3 mm.
in diameter; stigmas 3.

PERU: thickets and open woods, alt. 1800 to 2400 m., Huacapistana, dept.
Junín, June 5–8, 1929, *E. P. Killip & A. C. Smith 24182* in Herb. C. N. H.
(Field) M. 616242, TYPE.

This species resembles *Mauria ovalifolia* Turcz. in the character of the
inflorescence. It appears to be most closely allied to *M. sericea* Loes. from
which it differs in several minor details but from which it may at once be
distinguished by the very much smaller inflorescence and the five to seven
leaflets in *M. sericea*.

Mauria Cuatrecasasii[3] Barkley, sp. nov.   Arbor circa 8 m. alta; remulis
crassis, striatis, dense ferrugineo-pilosulis; foliis impari-pinnatis 6-jugis,
circa 45 cm. longis, dense ferrugineo-pilosis, foliolis supra sparse pilosis vel
subglabratis; petiolis circa 9 cm. longis; foliolis membranaceis, 9 mm. peti-
olulatis, anguste ovatis, integris, acuminatis, ad basem cuneatis vel late
cuneatis et decurrentibus, 35–45 mm. latis, 80–130 mm. longis; paniculis
axillaribus, crassis, pilosis; bracteis deltoideis, caducis; lobis calycis del-
toidis, glabris; drupa ovoidea, circa 7.5 mm. longa.

Tree to 8 m. high; branchlets thick, striate, densely ferrugineously short
pilose; leaves imparipinnately compound, about 45 cm. long, densely ferrugi-
neously velvety-pilose throughout except upper surface of leaves sparse-
pilose to subglabrate; petiole about 9 cm. long; leaflets about 13, membra-
naceous, short petiolulate with petiolules about 9 mm. long, narrowly ovate,
entire, acuminate, at the base cuneate to broadly cuneate and decurrent on
the petiolules, 35 to 45 mm. broad, 80 to 130 mm. long; panicles axillary,
coarse, pilose; bracts deltoid, caducous; calyx lobes deltoid, glabrous; fruit
ovoid, about 7.5 mm. long.

COLOMBIA: "palo Hernández," 200 m. alt., Cuesta de Fusagasugá, Dept.
Cundinamarca, 18 II 1940, *J. Cuatrecasas 8062* in Herb. U. S. Nat. 1796044,
TYPE.

This species shows affinities to *Mauria ferruginea* Tul. from which it
differs in having much longer leaves, membranaceous leaflets, and a less
dense white pilosity which is not confined to the nerves and veinlets. It more
closely resembles *M. sericea* Loes. which has subsessile, acute leaflets with
prominent veinlets and with ferrugineous pilosity in that species confined
to the midrib, nerves, and veinlets on the under surface.

[3] For J. Cuatrecasas.

Fig. 1. Type sheet of *Mauria Killipii* Barkl., Huacapistana, Peru, 5–8, June 1929, *Killip & Smith 24182* in Herb. Chic. Nat. Hist. (Field) Mus. 616242. Fig. 2. Type sheet of *Mauria Cuatrecasasii* Barkl., Departamento Cundinamarca, Colombia, 18 February 1940, *Cuatrecasas 8062* in Herb. U. S. Nat. 1796044. Fig. 3, Type sheet of *Mauria Dugandii* Barkl., Departamento Cundinamarca, Colombia, 26 January 1944, *Garcia-Barriga 11021* in Herb. U. S. Nat. 1852216.

Mauria Dugandii[4] Barkley, sp. nov.   Arbor circa 3 m. alta; ramulis crassis, lenticellis prominentibus; foliis circa 25 mm. longis, imparipinnatis, 2–3-jugis, senescentibus glabris, foliolis subtus exceptis; petiolis circa 6.5 cm. longis; foliolis lateralibus 2 mm. petiolulatis, foliolis terminalibus 2 cm. petiolulatis, elliptico-lanceolatis, nervis lateralibus atque venis utrinque dense reticulatis prominentibus, subtus valde caduce ferrugineo-pilosis; paniculis axillaribus, sessilibus, sparse pilosis, 6–12 cm. longis; bracteis caducis; drupa oblique oblongo-ovoidea, circa 10 mm. longa, 6 mm. lata, 3 mm. crassa.

Tree about 3 m. high; branches thick, brown, with prominent lenticels; leaves about 25 mm. long, imparipinnately compound, glabrate at maturity except for under surface of leaflets; petiole about 65 mm. long, leaflets about 5, subcoriaceous, lateral leaflets short-petiolulate with petiolules about 2 mm. long, terminal leaflet with petiolule about 2 cm. long, elliptic-lanceolate, with a reticulum of veinlets showing prominently, acuminate at apex, cuneate to rounded at base, glabrous above, fugaciously pilose on the nerves and veinlets below; panicles axillary in the axils of the uppermost leaves, sessile, sparsely pilose, 6 to 12 cm. long; bracts soon deciduous; drupes obliquely oblong-ovoid and somewhat flattened, about 10 mm. long, 6 mm. broad, red.

COLOMBIA: alt. 1900–2100 m., El Tablazo entre Subrachoque y San Francisco, finca "El Carmero," San Francisco Córdillera Oriental, Dept. Cundinamarca, 26 I 1944, *H. García-Barriga 11021* in Herb. U. S. Nat. 1852216, TYPE; alt. 1300 m., La Cabuya, región del Sararc, Departamento Norte de Santander, Cordillera Oriental, 14 X 1941, *J. Cuatrecasas, R. E. Schultes & E. Smith 12207* in Herb. U. S. Nat. 1850916.

This species resembles *Mauria puberula* Tulasne which has coriaceous leaflets, rachis, pilose, and long acuminate, lanceolate-ovate leaflets. It more closely resembles *Mauria ferruginea* Tulasne, which from a study of a photograph of the type appears to have smaller, more acuminate, ovate leaflets, and the pubescence of the rachis and petiole is not fugacious. The rachis and branches of the inflorescence (much smaller) of that species are permanently pilose.

From a study of the fragment and photograph of the type (*Karsten, prov. Bogota, Colombia*), it would appear that *Mauria ferruginea* Tul. var. *obtusifolia* Engler (in DC., *Monog. Phaner.* **4**: 331. 1883) would be better treated as a species, **Mauria obtusifolia** (Engler) Barkley comb. nov., the description for which should be amplified to include: leaves simple (above) to 7-foliolate, 67 to 130 mm. long; leaflets broadly elliptic, obtuse at apex, very broadly cuneate at base, ferrugineously pilose on young branches, petiole, rachis, and on the under surface of leaves and leaflets on the midrib, nerves, and veins; panicle small, densely pilose on the rachis and its branches.

CHICAGO NATURAL HISTORY MUSEUM HERBARIUM
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      AND
UNIVERSITY OF TEXAS HERBARIUM
   AUSTIN, TEXAS

[4] For Armando Dugand G.

NOTEWORTHY PLANTS OF SOUTH AMERICA—IV.
THREE VARGAS SPECIMENS[1]

Paul C. Standley and Fred A. Barkley

Among a series of specimens studied which were collected by C. Vargas C., were four specimens representing three species apparently undescribed. Descriptions of these follow.

Cajophora Vargasii Standley & Barkley, sp. nov. Herbacea scandens; caulibus tortuosis, gracilibus; petiolis circa 20 mm. longis, setosis, pilosis; laminis deltoido-lanceolatis, supra sparse et subtus dense setuloso-pilosis, 35–95 mm. longis, 18–42 mm. ad basim latis, breviter, acuminatis, serrato-lobatis, lobis serratis; pedunculis gracilibus, tortuosis, circa 10 cm. longis, pilosis; sepalis 5, deltoido-lanceolatis, serratis, pilosis, circa 13 mm. longis, 5 mm. ad basim latis; petalis cymbiformibus, 30 mm. longis, 15 mm. latis, ad nervum villosulis, squamis saccatis, convexis, rotundo-ovatis, 5 mm. longis, 4 mm. latis, venis 3, appendicibus 3, filiformibus, circa 3 mm. longis, 0.7 mm. ad basim latis; staminodiis filiformibus ad circa 13 mm. longis, 1 mm. ad basim latis; staminibus circa 80; capsulis piloso-setosis.

Setulose vine with slender, brownish, tortuous stems; petioles about 20 mm. long, setose, pilose; leaf-blades deltoid-lanceolate, setulose-pilose above and densely so on under surface, 35–95 mm. long, 18–42 mm. broad near base, short acuminate, serrately lobed from base, lobes obscurely serrate; peduncles slender, tortuous, about 10 cm long, appressed pilose; sepals 5, deltoid-lanceolate, serrate, pilose, about 13 mm. long, 5 mm. broad at base, petals cymbiform, 30 mm. long, 15 mm. wide, villosulous especially on the veins, whitish; scales yellowish, saccate, convex, rotund-ovate, 5 mm. long, 4 mm. wide, 3-nerved, bearing dorsally near the apex 3 filiform appendages about 3 mm. long and 0.7 mm. wide at their base, staminodia filiform to about 13 mm. long, 1 mm. broad at base; stamens about eighty; filaments slender, about 25 mm. long; anthers black, ovoid, about 1 mm. long; capsule twisted, pilose-setose.

Peru: "en un bosque de *Polylepis, Schinus, Eugenia*, etc.," 3600 meters, Departamento Cuzco, Provincia Paruro, 19 I 1942, *C. Vargas C. 2392* in Herb. Chic. Nat. Hist. (Field) Mus., Type.

A second specimen, *C. Vargas C. 2657* (from open forest, 2800 meters altitude, Tuncapata, Santa Rita, Dep't Cuzco, Prov. Urubamba, Peru, in Herb. Chic. Nat. Hist. (Field) Mus.) is apparently conspecific; it is almost identical in foliage, but the flower parts are about two-thirds as large. A third specimen, *Benj. Styles*, 18 Aug 1945 (in Herb. Chic. Nat. Hist. (Field) Mus.) apparently belongs here also.

This species is very similar to *Cajophora tenuis* Killip, but is at once distinguishable by the hispid capsule of that species. It is probably closest in relationship to *C. macrantha* Killip, but the leaves of that species are

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[1] Publication is partly at the expense of Fred A. Barkley.

hispid, the lobes more sharply dentate, the lobing is much deeper, the flowers are larger, and the glands are without the dorsal appendages.

Sicyos Vargasii Standley & Barkley, sp. nov.   Herbacea scandens, caule gracili striate sparse caduceque pilosus, internodiis elongatis; folia petiolata membranacea, orbicularia, albido-papillosa, petiolo crasso circa 5 cm. longo; lamina 6–9 cm. longa et subaequilata profunde quinquelobata; basi profunde cordata; inflorescentia pedunculata, floribus numerosis racemosis densis, pedicellis gracilibus ad 6 mm. longis; calyxibus 5-lobis; petalis 5; staminibus connatis, antheris contortis; fructibus immaturis fugaciter pilosis.

Sprawling, tendrilled, succulently herbaceous vine; stems soon glabrous, striate; tendrils 1- to 2-parted; petioles coarse, to about 5 cm. long, soon glabrous; leaf-blades 6–9 cm. long, thinly membranaceous, ovate in outline and 5-lobed with lobes acutish except the terminal lobe acuminate to (as is the commoner case) orbicular and twice lobate with lobe apex rounded-truncate to subacute, base cordate with a narrowly U-shaped sinus, short-white-papillose, puberulent on veins; peduncle of raceme to 10 cm. long, slender, fugaciously pilose, pistillate flowers clustered with staminate; perianth of pistillate flowers reduced; calyx of staminate flowers about 5 mm. broad, white, deeply 5-lobed; petals 5; filaments connate, anthers sessile, connate, flexuous; ovary ovoid, fugaciously pilose.

PERU: "Putac-llanco," annual vine among herbaceous plants on rocky slopes, near Asuncion Bridge, Rio Apurimac, Depto. Cuzco, Prov. Canas, Feb. 28, 1939, *C. Vargas 11025* in Herb. Chic. Nat. Hist. (Field) Mus., TYPE.

Loasa Raimondii[2] Standley & Barkley, sp. nov.   Herba annua (vel perennans?); caulibus pilosis hispidulosisque, striatis, internodiis circa 7 cm. longis; foliis simplicibus, petiolatis; petiolis hispidulis, circa 1 cm. longis; laminis hispidulis pilosisque, 10–20 mm. latis, 45–30 mm. longis, irregulariter sinuato-dentatis, late lanceolatis, acutis, ad basim angustis inflorescentiis foliatis-racemosis; pedicellis circa 18 mm. longis; sepalis viridibus, ovatis, acutis, pilosis setosisque, circa 4 mm. longis; petalis planis, membranaceis, albis, obovatis, subacutis, circa 10 mm. longis; squamis inflatis, 2 mm. latis, 3 mm. longis; staminibus multis, fasciculatis, circa 6 mm. longis; capsulis circa 20 mm. longis, 5 mm. latis, hispidis pilosisque.

Subscandent or sprawling, coarse annual (or perennial?); stem yellowish, pilose, setose, striate, internodes about 7 cm. long; leaf simple, petiolate; petiole hispid and pilose, about 1 cm. long; blade sparsely setose and sparsely pilose, 10–20 mm. broad, 45–30 mm. long, irregularly sinuate-dentate, broadly lanceolate, acute, narrowed toward base, abruptly truncate at base, rarely ovate and 5-lobate; inflorescence a leafy subscorpioid raceme; flower large; pedicels not adnate to the axis, about 18 mm. long in fruit; sepals green, ovate, acute, pilose and setose, about 4 mm. long; petals white, obovate, subacute, about 10 mm. long, clawed, pilose and setose, spinose-ciliate; scales inflated, scarcely saccate at base, 2 mm. wide, 3 mm. long; stamens many, fasciculate and opposite the petals, about 6 mm. long; capsule about 20 mm. long, 5 mm. broad, subobconical, hispid and pilose, 3-valved, the valves alternating with the sepals; seeds many, rough, angular, about 1 mm. in diameter.

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[2] Honoring Antonio Raimondi.

Fig. 1. Type sheet of *Loasa Raimondii* Standl. & Barkl., Departamento Cusco, Peru, 28 March 1942, *Vargas 2672* in Herb. Chic. Nat. Hist. (Field) Mus. 1164358. Fig. 2. Type sheet of *Sicyos Vargasii* Standl. & Barkl., Departamento Cusco, Peru, 28 February 1929, *Vargas 11025* in Herb. Chic. Nat. Hist. (Field) Mus 1164359. Fig. 3. Type sheet of *Cajophora Vargasii* Standl. & Barkl., Departamento Cusco, Peru, 19 January 1942, *Vargas 2392* in Herb. Chic. Nat. Hist. (Field) Mus. 1164356.

PERU: Along ancient ruins, 2100 meters altitude, Hacienda Tuncapata Stihit, Departamento Cuzco, Provincia Urubamba, 28 III 1942, *C. Vargas C. 2672* in Herb. Chic. Nat. Hist. (Field) Mus., TYPE.

This species appears to be related to *Loasa carunculata* Urb. & Gilg which has densely pilose-setose, more lanceolate, and coarser leaves and much larger flowers with orange petals, and to *L. picta* Hook. f. which also has orange petals, shorter sepals and petals, broader leaves, and on which the capsules are cylindrical and abruptly tapered at the base.

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NOTEWORTHY PLANTS OF SOUTH AMERICA—V.
THE GENUS OCHOTERENAEA

FRED A. BARKLEY

At the time the monotypic genus *Ochoterenaea* was published[1] it was known from a single fruiting collection. From its compact thyrsus of fruits, sufficient remnants of flowers were available to furnish some knowledge of their structure.
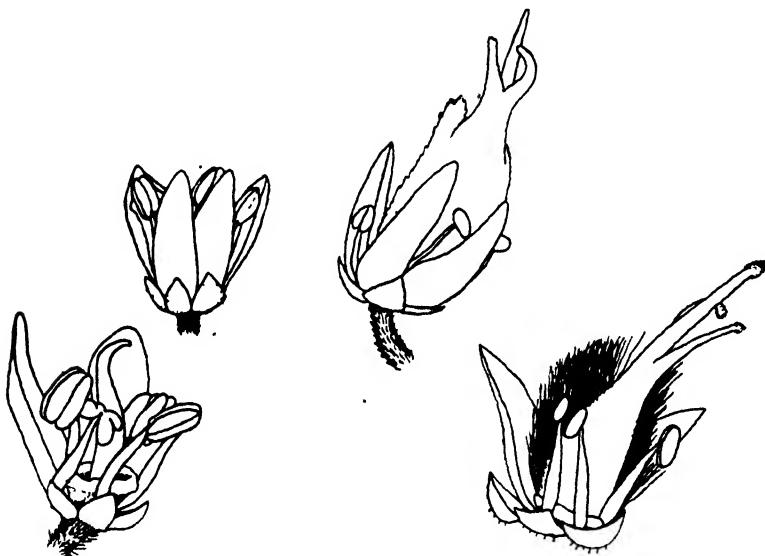
FIG. 1. Camera lucida sketches of flowers of *Ochoterenaea columbiana* Barkley. The upper from dried material, the lower boiled material with some petals removed to better show the flower parts. The staminate flowers to the left from *Garcia 10748*, the pistillate flowers to the right from *Steyermark 56745*. Note the mat of glandular substance obscuring the marginal hairs in the dried specimen of pistillate flower. Drawings × 12.

Additional recent collections of the plant have been made so that further knowledge of its distribution and the structure of its flowers is available.

The specimens of *Ochoterenaea columbiana* Barkley which have been examined are as follows.

VENEZUELA: state of Mérida: "cedrillo," tree 30 feet tall, flowers dull whitish, peduncle and calyx pale green, leaves membranous, grass-olive green above and pale green below, juice in cut stem milky, fruit pale green with

[1] Bull. Torrey Club **69**: 442–444. 1942.

lavender silky margin, ovary fragrant-spicy, rich forested slopes along Río Onia, near Bolero, north of Mesa Bolívar, alt. 550–915 mtrs., 23 May 1944, *Julian A. Steyermark 56745* [Herb. Chicago N. H. (Field) Museum, Herb. U. Texas]. COLOMBIA: department El Valle: dense forest, Rio Digua Valley, between La Elsa and Rio Blanco, alt. 725 mtrs., 2–5 April 1939, *E. P. Killip 34772* (Herb. U. S. Nat., TYPE); department Cundinamarca: arbol mas menos 4 mtrs., La Vega, Camino a Nocaima, alt. 1200–950 mtrs., 27–29 Enero 1942, *H. Garcia Barriga 10640* (Herb. U. S. Nat.); entre Pacho y Rio Negro, carretera y alrededores, alt. 1000–1200 mtrs., 22–26 Febrero 1942, *H. Garcia Barriga 10748* (Herb. U. S. Nat.).

Killip 34772 has mature fruits, *Garcia 10640* has immature fruit, *Steyermark 56745* contains pistillate flowers, immature fruit, and mature fruit, while *Garcia 10748* has staminate flowers. The specimens indicate that the species is remarkably constant over its known range, as only minute variations were noted between the specimens. Sketches of staminate and pistillate flowers accompany this note (fig. 1).

DEPARTMENT OF BOTANY AND BACTERIOLOGY, UNIVERSITY OF TEXAS
    AUSTIN, TEXAS

TORREYA

An Apparently New Record of Globulinea gigantea Ulke. Dr. Curt
Teichert, of the University of West Australia, Crawley (Perth), Australia, has
figured what appears to be *Globulinea gigantea* Ulke in figure 4 of an article in
Bull. Am. Assoc. Petroleum Geologists **25**(3): 385. Mr 1940. This fossil alga has
hitherto been found only in Indiana limestone. Dr. Teichert's specimen was found
in the Kimberly District of northwestern Australia in ferruginous sandstone
(Liveridge Series) of Upper (Permian) Paleozoic Age. He called it a "Large
Gastropod Trail." This diagnosis seems inapplicable, however, for a marine gastro-
pod trail would evidently show only on the surface and would hardly extend into
sandstone rock, originally mud of course, as illustrated. These gastropod trails, or
mud-eating *Nereites* tracks, appear to be five-eighths of an inch across judging by
the size of the geologist's hammer shown in the picture, and this agrees with the
size of the axis of *Globulinea gigantea* Ulke.—TITUS ULKE.

PROCEEDINGS OF THE CLUB

Minutes of the Meeting of May 15, 1946. Dr. Zimmerman called the regular meet-
ing of the Torrey Botanical Club to order at 3:30 p.m. in the members room of the New
York Botanical Garden with 18 members and guests present. The minutes of the previous
meeting were approved as read. Eight new associate members and three annual members,
already approved at the Council meeting, were elected by the members.

The Club approved a resolution to have a memorial written for the late Dr. Robert
Almer Harper.

The scientific portion of the day's program was a paper presented by Dr. J. S.
Karling on: ''Keratinophilic Chytrids.''

The meeting adjourned at 4:30 p.m.

Respectfully submitted,
LIBERO AJELLO
Recording Secretary

Minutes of the Meeting of October 1, 1946. Dr. Zimmerman opened the first fall
meeting of the Torrey Botanical Club at 8:15 p.m. at the Brooklyn Botanic Garden.
Twenty members and friends were present. The minutes of the previous meeting were
approved as read.

Dr. Simpson was in receipt of a letter from Cuba requesting information on the
relation of *Chara* to mosquito control. The letter was turned over to Dr. Johnson, who
was to refer it to the Department of Entomology, Rutgers University.

No further business was transacted and the meeting was opened to an informal dis-
cussion of the summer activities of the members.

The meeting adjourned at 9:45 p.m., refreshments being served by the staff of the
Brooklyn Botanic Garden.

Respectfully submitted,
LIBERO AJELLO
Recording Secretary

**Report of the Committee to Prepare a Memorial for the late Dr. Robert Almer
Harper. October 16, 1946.** As an appropriate feature of the celebration of the Seventy-

fifth Anniversary of the Founding of The Torrey Botanical Club, Volume 69, 1942, of the BULLETIN was dedicated to Robert Almer Harper and Herbert McKenzie Denslow. The May number of that volume, containing a brief biographical sketch, was specifically designated as the ''Harper Number.'' Today The Torrey Botanical Club again pays its respects, through this Memorial, to Professor Harper, who died at his home near Bedford, Virginia, May 12, 1946.

Robert Almer Harper was elected to membership in the Club October 10, 1911. From his boyhood days at Port Byron, Illinois, he had been greatly interested in the plants of the regions wherever he lived. On coming to New York he continued this interest especially in the local Cryptogamic Flora. It was only natural that soon after he became President of the Club January 13, 1914, he should ask permission to appoint a number of persons to be Chairmen of Special Committees on Local Flora. Accordingly he appointed twenty-four such chairmen, and he himself undertook to do special work on the genus *Cortinarius*. This list of Chairmen, with necessary changes, has been continued and published in TORREYA.

Professor Harper strongly supported the movement to establish a class of member ship to include those to be known as Associate Members. He was one of the first to become a Sustaining Member and he maintained this membership until his death.

When we read the Proceedings of the Club and News and Notes published in TORREYA since 1911, we are made to realize how wide were his interests in the plant sciences and his many contributions to the welfare of the Club. By his death Botany lost a great investigator and teacher, the Club lost one of its most enthusiastic supporters, and his colleagues a good friend and counselor. Therefore,

Be it resolved that this memorandum be recorded in the minutes of this meeting and published with the Proceedings of the Club. It is also directed that copies of these minutes be transmitted to Mrs. Harper and other members of the family with expressions of the deepest sympathies of the members of the Club.

(Signd) B. O. DODGE
JOHN S. KARLING
H. W. RICKETT

NEWS NOTE

The following appeal was sent to the President of the Club through the Office of Foreign Relations, Manila:

''At the outbreak of World War II, the Scientific Library of the Bureau of Science, an office under this Department, had one of the largest and best known collections of technical and scientific publications in this part of the Orient. This same library was destroyed by the Japanese during the war.

''We shall appreciate it, therefore, if you will kindly help us in the task of building anew from scratch some such collection by donating to the library whatever publications you can spare now and in the future. Please send them addressed to: Scientific Library, Bureau of Science, Manila, Philippines.''

(Signed) JOSE S. CANUS
Under Secretary

INDEX TO AMERICAN BOTANICAL LITERATURE

COMPILED BY
LAZELLA SCHWARTEN
WITH THE COLLABORATION OF THE EDITOR OF THE TAXONOMIC INDEX

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used to include the entire Western Hemisphere.

Papers that relate exclusively to bacteriology, forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included. If users of the Index will call the attention of the Bibliographer to errors or omissions, their kindness will be appreciated.

The Index is reprinted monthly on cards, and furnished in this form to subscribers at the rate of three cents for each card. The different subjects as classified below may be ordered separately (but no orders will be taken for less than one year's issue in any classification). Correspondence relating to the card issue should be addressed to the Treasurer of the Club.

TAXONOMY, PHYLOGENY AND FLORISTICS

ALGAE

(See also under Morphology. Hall; under Physiology. Smith.)

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DISTRIBUTION AND NATURE OF POLYPLOIDY IN
·.FESTUCA ELATIOR L.[1]

W. M. MYERS AND HELEN D. HILL[2]

In the past the plant breeder has dealt in most cases with species all or nearly all plants of which had a constant chromosome number. Rare deviations which occurred were hypo- or hyperploids or polyploids that arose in experimental material. In many species of the plant kingdom, however, races with different chromosome numbers have been reported as occurring naturally. The phenomenon of intraspecific chromosome races was considered in detail by Müntzing (1936) who concluded that more than 100 cases were known in the Angiosperms.

In a recent survey of the literature of *Gramineae*, Myers (in press) found 99 grass species in which two or more intraspecific chromosome races had been reported. This number does not include several species, such as *Poa pratensis*, in which apomixis occurs and results in perpetuation of aneuploid chromosome numbers nor species in which the variability may be conditioned by the high degree of polyploidy, as in *Alopecurus alpinus* and *A. antarcticus*. Among the species with intraspecific chromosome races are some of the most important forage grasses including *Phleum pratense*, *Bromus inermis*, *B. marginatus* (*B. carinatus*), *Festuca elatior*, *Agropyron cristatum*, *A. smithii*, *Phalaris arundinacea*, *Andropogon furcatus*, and others. An extreme case of intraspecific polyploidy has been reported in *Panicum virgatum* by Nielsen (1944) who found 2n numbers of 18, 36, 54, 72, 90, and 108 chromosomes (2x, 4x, 6x, 8x, 10x, and 12x). Among 17 collections from an area of not more than 10 acres near Chippewa Falls, Wisconsin, 2x, 4x, 6x, 8x, and 10x forms were found.

Intraspecific polyploidy is of frequent occurrence in the genus *Festuca*, the condition having been found in six species and particularly in *F. elatior*, *F. rubra*, and *F. ovina*. In *F. elatior*, the diploid chromosome number of 14 was reported for European material by Kattermann (1930), Litardière (1923), and Stolze (1925), and for North American collections by Church (1936) and Nielsen and Humphrey (1937). Somatic numbers of 14 and 42 were reported by Nakajima (1930, 1931) and Evans (1926). The most detailed investigations of chromosome number in *F. elatior* were those of

Levitskii and Kuzmina (1927) and Stähliu (1929). Their results are sum-marized in table 1.

TABLE 1. *Chromosome numbers in Festuca elatior according to Levitskii and Kuz-mina (L&K) and Stähtin (S).*

Subspecies and Variety	2n Chromosome Number	Authority
Subspecies *pratensis*		
var. *genuina* Hack. (var. *eu pratensis* St.Y.)	14	L&K, S.
var. *apennina* Hack.	42	S.
Subspecies *arundinacea* Hack.		
var. *genuina* Hack. (var. *eu-arundinacea* St.Y.)	42	L&K, S.
var. *Fenas* Hack. (*glaucescens* Boiss.) subvar. *corsica* Hack.	42	L&K.
var. *Uechtritziana* (Wiesbaur) Hack.	14, 28	S.
var. *Letourneuriana* St.Y. subvar. *Pitardii*	70	L&K.
var. *Cirtensis* St.Y.	70	L&K.

According to Levitskii and Kuzmina (1927) these cytologically different races are frequently difficult to distinguish morphologically and can be sepa-rated accurately only by determination of the chromosome numbers. In North America, Hitchcock (1935) recognized two forms, *Festuca elatior* L., meadow fescue, and *F. elatior* var. *arundinacea* (Schreb.) Wimm., tall fescue. These two forms are considered by agronomists to be adapted to some-what different conditions, tall fescue, although tolerant of moist conditions, being more drouth resistant and better adapted to infertile soils than meadow fescue. When grown under comparable conditions in field plots, as spaced plants in the breeding nursery, or in greenhouse pots, the tall fescue plants are in general rather readily distinguishable from meadow fescue by their greater height, more robust appearance, and the greater size of vegetative and flower parts. Furthermore, it has been found (Kreitlow & Myers, in press) that meadow fescue plants are predominantly susceptible and tall fescue plants predominantly immune or resistant to crown rust, *Puccinia coronata*. Because of the importance of a knowledge of chromosome numbers and interrelationship of plants to be used in a breeding program, a cyto-logical study of material collected from natural habitats and of existing strains of tall and meadow fescue was conducted.

COLLECTIONS FROM NATIVE STANDS

Festuca elatior is a native of Eurasia introduced into North America, and is now widely distributed in much of the northeastern region, occurring particularly in moist locations along roadsides, in lowland pastures, and occasionally in higher and somewhat drier locations. During the summer of 1941, individual panicles were collected from 24 locations in Centre County, Pennsylvania, and the chromosome number was determined of one plant

obtained from seed from each panicle. The chromosome numbers were deter-
mined from root-tips prepared for study by the methods described by Hill
and Myers (1945).

Fourteen collections (a total of 166 single panicles) were obtained from
the Spring Creek drainage area in the vicinity of State College. Nine of these
collections (94 panicles) were from roadsides and permanent pastures ad-
jacent to and subject to overflow from the creek during floods. All plants
examined were diploid, 2n = 14, and, when grown in the nursery, were classi-
fied as meadow fescue types. Five collections (72 panicles) were from dry
hillside locations. In general, the distribution of fescue plants was continu-
ous, the stand becoming thinner with increased distances from Spring Creek.
In four of these collections, all of the plants examined were diploid, 2n = 14,
and meadow fescue type. One collection of 20 panicles was made in an area
separated from the population of fescue adjacent to Spring Creek by a road
and farmstead.[3] All of the plants examined from seed obtained from these
panicles were hexaploid, 2n = 42, and in general could be classified in the
breeding nursery as tall fescue types. Some plants, however, were not dis-
tinctly different in size and robustness from meadow fescue.

Four collections, two (19 panicles) from high ground and two (21 pani-
cles) from low wet locations in the area east and north of Centre Hall,
Pennsylvania, produced only diploid, 2n = 14, plants of meadow fescue type.
Similarly a collection (12 panicles) near Potters Mills, Pennsylvania, and
one (15 panicles) near Boalsburg, Pennsylvania, both from low areas, were
diploid, as also were two collections (23 panicles) from high, dry locations
and one (14 panicles) from a bottomland pasture near Stormstown, Penn-
sylvania. A single plant found in a clearing on the summit of Tussey Moun-
tain above Pine Grove Mills, Pennsylvania, was hexaploid.

It is apparent from these results that both diploid and hexaploid types
of *Festuca elatior* occur in natural stands in central Pennsylvania, the
diploid, however, being considerably more common. Only fragmentary infor-
mation is available regarding the distribution of these forms elsewhere in
this country. Church (1936) and Nielsen and Humphrey (1937) reported
only diploids in their studies. In the course of the present investigations,
three collections of panicles from near Orono, Maine, were received from
Doctor F. H. Steinmetz, and all plants examined from the seed were diploid.
A fourth collection, obtained two years later by Doctor Steinmetz from one
of the same meadows, proved to be uniformily hexaploid.

CHROMOSOME NUMBERS OF VARIETIES

Twenty plants were established from each of seven varieties of *Festuca
elatior* included in the Uniform Grass Nurseries and the chromosome num-

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bers were determined from root tip preparations.[4] In the following four
varieties, classified as meadow fescue types, all plants were diploid:

1. English grazing strain, S–53, from the Welsh plant Breeding Station, seed
   increased at the Soil Conservation Service Nursery, Big Flats, New York.
2. Svalöf Early Meadow Fescue, seed increased at the Massachusetts Agricultural
   Experiment Station.
3. Otofte, seed increased at the Massachusetts Agricultural Experiment Station.
4. Minnesota No. 1449, selection from northern grown commercial seed.

In the following three varieties, classified as tall fescue types, all plants were
hexaploid:

1. No. 2659 from Soil Conservation Service Nursery, Ithaca, New York, the seed
   originally obtained from England.
2. Alta fescue, F. C. 29,366, developed at the Oregon Agricultural Experiment
   Station.
3. Kentucky No. K–31, developed at Kentucky Experiment Station.

MEIOSIS IN DIPLOID AND HEXAPLOID TYPES

Meiotic behavior was studied in the microsporocytes of three diploid and
six hexaploid plants. The material for study was collected from plants grown
in greenhouse pots and was fixed in acetic alcohol. All data were collected
and photomicrographs made from fresh aceto-carmine smear slides.

The diploid plants were generally regular in meiosis (table 2). Seven
bivalents were found in most sporocytes at diakinesis and metaphase I but

TABLE 2. *Meiosis in diploid (2n = 14) plants of Festuca elatior.*

Plant No.	Diakinesis		Metaphase I		Anaphase I			Quartets	
						Per cent with			
	No. cells	X-ta per chromosome	No. cells	% with univalents	No. cells	Laggards	Bridge + fragment	No. cells	% with micronuclei
41–3 (3)	20	1.76	209	7.2	126	0.8	2.4	226	3.5
41–130 (5)	12	2.02	230	0.4	95	1.0	0.0	135	0.0
41–210 (4)	12	2.04	142	5.6	108	5.6	0.0	111	0.9

occasional sporocytes were observed with six bivalents and two univalents
(figs. 1, 2, 3). Likewise the incidence of lagging and equationally dividing
univalents at anaphase I and of micronuclei in the quartets was relatively
low. In these characteristics and also in chiasma frequency the plants were

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[4] The authors are indebted to Mr. M. A. Hein, Senior Agronomist, Division of Forage
Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agri-
cultural Research Admin., U. S. Department of Agriculture, for this seed and for infor-
mation of the origin of the varieties.

similar in behavior to diploid plants of *Lolium perenne* (Myers 1941).
Dicentric chromatid bridges and acentric fragments were observed in one
plant (fig. 4), indicating heterozygosity for an inversion in this plant

At diakinesis in the six hexaploid plants (figs 5, 6), the average fre-
quency of bivalents per sporocyte ranged for the different plants from 16.2
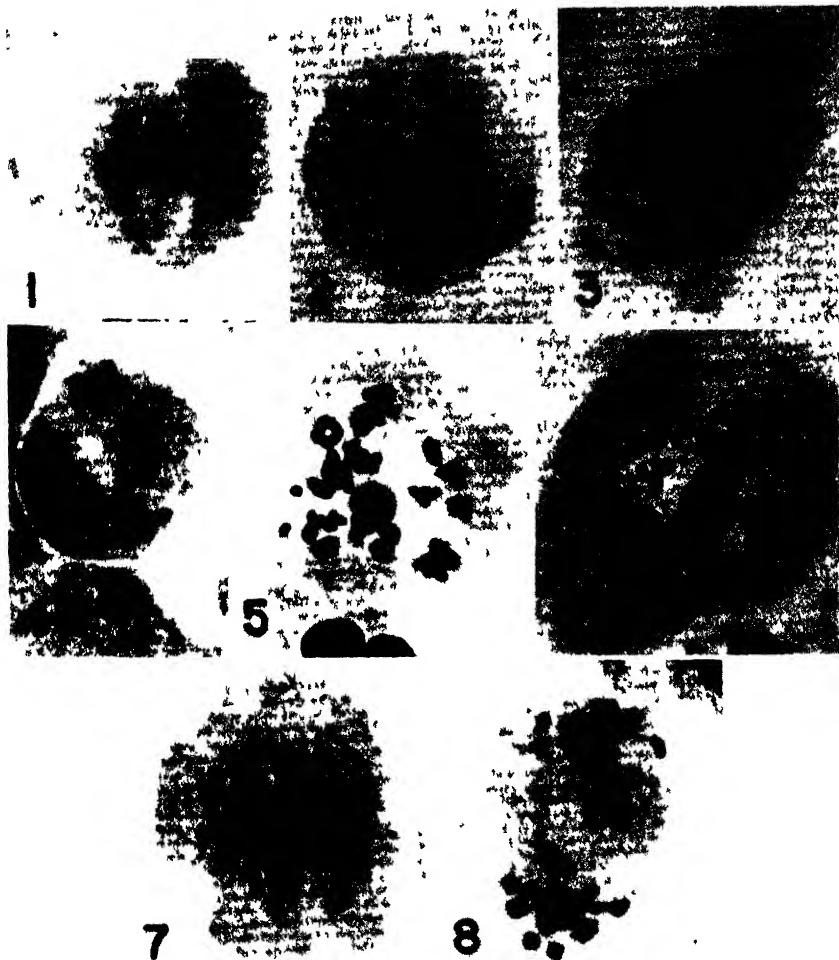
FIGS. 1–8 Meiosis in diploid and hexaploid races of *Festuca elatior* L. ×ca. 850.
FIGS. 1, 2. Metaphase I in the diploid race with 7 bivalents. FIG 3. Metaphase I in
diploid race with 6 bivalents and 2 univalents. FIG 4. Anaphase I in diploid race showing
dicentric chromatid bridge. FIG 5 Diakinesis in hexaploid race showing two unpaired
centric fragments. FIG. 6 Diakinesis in hexaploid with two centric fragments associated
by a single chiasma. FIG. 7. Metaphase I in hexaploid race showing paired but non-
oriented centric fragments. FIG. 8. Anaphase I in hexaploid race with two lagging and
dividing univalents

to 19.8 while the average frequency of quadrivalents ranged from 0.6 to 2.8. The maximum number of quadrivalents in the sporocytes examined was 3 in 38–2(1), 4 in 38–12(2), 3 in 38–12(1), 5 in 38–1(1), 3 in 38–11(4), and 2 in 1407. Thus in no case was the theoretically possible maximum of seven quadrivalents observed. Sexivalents were observed in only one plant, 38–12(2), one sexivalent occurring in each of three of the six sporocytes analyzed. The results obtained in these investigations differ from those reported by Peto (1933) who found no multivalent association in *F. arundinacea* (2n = 42). Data on chromosome association in relation to homologies must be interpreted with caution since factors other than lack of homology may be responsible for absence of pairing, particularly of multivalent association. Nevertheless, there is no positive evidence from these data of complete homologies between chromosomes from different genomes.

The hexaploid plants varied considerably (table 3) in frequency of metaphase I sporocytes with univalents, anaphase I with laggards (fig. 8), and quartets with micronuclei. In the limited data available, there was no evi-

TABLE 3. *Chromosomal association and behavior in stages of meiosis in hexaploid plants of Festuca elatior.*

| Plant No. | Diakinesis | | | | | | | | Metaphase I | | | Anaphase I | | | Quartets | |
| | Average per sporocyte of | | | | Half X ta per chromosome in | | | | | | % with | | | | |
	No. cells	I	II	IV	VI	II	IV	VI	Total	No. cells	% with univalents	No. cells	Laggards	Bridge + fragment	No. cells	% with micronuclei
38–2(1)	16	0.0	17.9	1.6	0.0	1.82	1.98		1.84	214	10.3	90	14.4	3.3	172	16.3
38–12(2)	9	0.0	14.4	2.8	0.3	1.92	2.06	1.88	1.96	222	2.7	136	3.7	23.5	170	23.0[a]
38–1(1)	10	0.0	17.8	1.6	0.0	1.76	1.90		1.78	203	4.9	14	14.3	0.0	221	7.7
38–12(1)	16	0.4	18.7	1.1	0.0	1.72	1.94		1.74	130	11.6	37	59.4	5.4	120	17.4
38–11(4)	8	0.0	16.2	2.4	0.0	1.72	1.82		1.76	203	1.0	64	4.7	21.9	160	4.4
1407	17	0.0	19.8	0.6	0.0	1.72	1.96		1.74	124	2.4	164	6.1	9.2	167	17.8

[a] The fragments were large in this plant and often could not be distinguished at this stage from the chromatids from Anaphase I laggards. This value, therefore, is for all micronuclei from both sources.

dence that the irregularities of metaphase and anaphase were correlated with incidence of multivalents at diakinesis. In this regard, the results are consistent with those obtained previously in *Dactylis glomerata*, *Phleum pratense*, and autotetraploid *Lolium perenne* (Myers 1943, 1944, 1945; Myers & Hill 1942, 1943).

In five of the six plants, dicentric bridges and acentric fragments were observed in some sporocytes at anaphase I. In the sixth plant only 14 ana-

phase I sporocytes were examined. Such configurations ordinarily result from crossing-over in heterozygous inversions and have been found commonly in plants of naturally cross-pollinated, perennial grass species (cf. Myers, in press). The bridges and fragments in these plants may also have arisen from crossing-over in inverted segments of normally homologous chromosomes. On the other hand, some or all of these configurations could have resulted from structural rearrangements in partially homologous chromosomes from different genomes that paired occasionally to form quadrivalents or, rarely, sexivalents. In this regard, it is interesting, although perhaps only coincidental, that the two plants with high incidence of bridges and fragments [38–11(4) and 38–12(2)] also had the highest frequency of quadrivalents.

OCCURRENCE AND BEHAVIOR OF CENTRIC FRAGMENT CHROMOSOMES

Supernumerary centric fragments have been reported in diploid plants of *Festuca elatior* by Rancken (1934). Similarly, a centric fragment, in addition to the normal complement of 14 chromosomes, was found in four diploid plants of which chromosome numbers were determined in the present studies. Meiotic investigations in these plants have not yet been conducted.

In one of the hexaploid plants, 38–12(1), two fragments with approximately median centromeres were observed at diakinesis and metaphase I. In twelve of sixteen sporocytes at diakinesis, the two fragments were associated by a single chiasma (fig. 6). In two sporocytes, the fragments were paired with a single chiasma and the other arm of one fragment was associated by a chiasma with a chromosome in one of the bivalents. In one sporocyte, one fragment was paired with a member of a normal quadrivalent while the other was unpaired. Both fragments were unpaired in only one sporocyte (fig. 5). At metaphase I, two unpaired fragments were observed in 9.2 per cent of the 130 sporocytes examined, while a single unpaired fragment occurred in 0.8 per cent of the sporocytes. In 28.5 per cent of the cells, the fragments were paired but were not oriented on the equatorial plane (fig. 7). In many but not all of the remaining cells, the paired fragments could be seen among the bivalents and quadrivalents on the equatorial plane. At anaphase I, one or two lagging and dividing fragments were observed in 27 per cent of the sporocytes. The excess of this value over the 10 per cent of unpaired fragments at metaphase I may have resulted from lagging of part of the non-oriented fragment pairs. Observations of early anaphase I indicated that the oriented pairs of fragments disjoined and moved towards the poles along with the normal chromosomes.

Janaki Ammal (1939) postulated that the supernumerary chromosomes found in *Sorghum purpureo-sericeum* were relics of an evolutionary process of reduction in basic chromosome number. The fragment chromosomes of

Festuca elatior, because of their tendency to pair with normal chromosomes, could result, on the other hand, in the production of genetically balanced gametes of altered karyotype consisting of one chromosome more than normal. With proper conditions of isolation, therefore, the fragments of this species might provide an opportunity for evolutionary increase in basic chromosome number.

HYBRIDIZATION OF DIPLOID AND HEXAPLOID RACES

In the hybridization studies, advantage was taken of the facts that the plants used as parents were nearly completely self-sterile and that the F_1 plants could be identified readily and accurately by their intermediate chromosome number. Hence, emasculation of the female parent was unnecessary and crossing was effected by bagging together, just before flowering, panicles from diploid and hexaploid plants. Seed obtained in this manner was planted individually in pots and the chromosome numbers determined from root-tip preparations. Plants with 28 chromosomes were judged to be F_1 hybrids since no other 28 chromosome *Festuca* plants have been found in investigations at the U. S. Regional Pasture Laboratory.

A total of 13 panicles of as many hexaploid plants was cross-pollinated with panicles from diploid and 10 diploid panicles were crossed with hexaploid. Diploid panicles used in the other three cross-pollinations were not saved since they had started to flower before bagging No F_1 plants were obtained when the hexaploids were used as the female parent. Similarly, none was obtained from seven of the diploid panicles used as females From three diploid panicles, however, F_1 plants were found; eight from one, and one from each of the others. Less than half of the seed from each of the three panicles was used. Assuming an equal frequency of F_1 among the unused seeds, the total calculated number of F_1 obtained from the three panicles were 17, 3, and 2, respectively. Although the number of florets involved was not determined, the results suggest considerable variation in cross-compatibility of the different parent plants.

The variation between reciprocals in this investigation is consistent with the results reported by Jenkin (1933) in hybridization of *Lolium* with *Festuca* and by Nilsson (1939) in hybridization of *F. pratensis* (= *F. elatior*, 2n = 14) × *F. arundinacea* (= *F. elatior* var. *arundinacea*, 2n = 42). These crosses, likewise, were usually most successful when the female parent had the lower chromosome number. Such results are different from those most commonly obtained in interspecific hybridization (cf. Watkins 1932; Thompson 1930, 1940; Müntzing 1933).

Clones of the F_1 plants were grown in the greenhouse in the winters of 1944–45 and 1945–46. One clone flowered both winters and three others flowered during the second winter. The clones were completely male-sterile,

not a single anther dehiscing. Furthermore, no seed was obtained on more than 20 panicles that flowered in close proximity to dehiscing plants of diploid *F. elatior*.

MEIOSIS IN INTER-RACIAL AND INTERSPECIFIC HYBRIDS

Chromosomal behavior during meiosis was studied in one F_1 plant of *Festuca elatior* (2x) $\times$ *F. elatior* (6x) and in one F_1 plant of *Lolium perenne* $\times$ *F. elatior* (6x). As can be seen in table 4, pairing at diakinesis was essentially similar in the two plants A majority of the chromosomes occurred

TABLE 4. *Chromosome pairing at diakinesis in F_1 hybrids of Lolium perenne $\times$ F. elatior (6x) and of F. elatior (2x) $\times$ F. elatior (6x).*

Plant No.	No. cells	Diakinesis							
		Average number of							Total x-ta
		I	II	X ta[c]	III	X-ta	IV	X-ta	
1[a]	16	1.25	8.44	1.60	0.60	1.60	2.00	1.54	1.50
2[b]	9	1.33	7.78	1 78	0.89	1.50	2.11	1.68	1.64

[a] Plant Number 1—F_1 of *Lolium perenne* $\times$ hexaploid *Festuca elatior*.
[b] Plant Number 2—F_1 of diploid *F. elatior* $\times$ hexaploid *F. elatior*.
[c] Half-chiasmata per chromosome.

as bivalents, with some paired as quadrivalents and, less frequently, as trivalents or unpaired. The chiasma frequencies were somewhat lower than in the diploid or hexaploid plants examined, indicating some interference with pairing or chiasma formation in the hybrid plants, caused perhaps by partial lack of homology between the chromosomes that paired.

The high frequency of bivalents, trivalents and quadrivalents in these hybrid plants indicates, however, considerable homology between chromosomes of different genomes in the hexaploid and between chromosomes of the diploid and of the hexaploid. In this respect, the behavior is consistent with that observed in the hexaploid plants (table 3) where quadrivalents and, rarely, sexivalents occurred The results agree fairly well with those reported by Peto (1933) for hybrids of *Lolium perenne* $\times$ *Festuca arundinacea* (*F. elatior* var. *arundinacea*). He found an average of 9.07 univalents, 7.73 bivalents, 0.40 trivalents, 0.40 quadrivalents, and 0.13 quinquevalents per sporocyte. In *F. pratensis* $\times$ *F. arundinacea* (*F. elatior*, 2x, $\times$ *F. elatior*, 6x), however, Nilsson (1939) reported that $7_{11} + 14_1$ were normally found in meiosis.

The similarity of chromosome pairing between *Lolium perenne* $\times$ *Festuca elatior* (6x) and *F. elatior* (2x) $\times$ *F. elatior* (6x) is of particular interest with respect to reported chromosomal behavior in F_1 plants of *L. perenne* $\times$ *F.*

elatior (2x). In these hybrids, Peto (1933) found 7 bivalents regularly formed with a chiasma frequency only slightly lower than that of the two parents, indicating rather complete homology between chromosomes of the two species. The present study provides further evidence of the similarities of the *Lolium* and *Festuca elatior* genomes so far as pairing relationships are concerned.

DISCUSSION

Cytogenetic studies of intraspecific chromosome races are of interest to the systematist, the cytogeneticist, and the plant breeder. Although determination of the taxonomic status of the diploid and hexaploid races was not within the scope of these investigations, certain of the results have considerable bearing on that problem. Under comparable and favorable conditions, such as in the plant breeding nursery and greenhouse, most plants of the two races are rather readily distinguishable by casual inspection. It seems probable that measurements of various plant organs would reveal significant and rather consistent size differences between plants of the races.

In the crossing experiments, F_1 seed and plants were obtained relatively infrequently even when compatible pollen of the female parent species was excluded. It is probable, therefore, that in nature F_1 hybrids would occur very rarely. Furthermore, results of these investigations indicate that the F_1 hybrid plants are nearly, if not quite, sterile. Hence, frequency of gene exchange between the two races, under natural conditions, would be extremely low. From these standpoints, the two races, with some justification, might be given specific rank as has been done in fact, by some European workers. From the standpoint also of their use as forage plants, there might be some justification for considering these races as different species. Many agronomists and plant breeders recognize that meadow and tall fescue are distinct crops, adapted to different conditions and uses.

The role of autopolyploidy in origin of new forms has been much debated. Müntzing (1936) summarized considerable information that seemed to provide evidence of the importance of autopolyploidy in this respect. The polyploid intraspecific chromosome races were considered by him to have arisen as autopolyploids and Darlington (1937) has expressed a similar opinion. The data from meiosis in the hexaploid *Festuca elatior* and in hybrids of hexaploid with diploid *F. elatior* and *Lolium perenne* indicate that this race does not behave cytologically as an autohexaploid at the present time. Pairing between chromosomes of the different genomes is not complete. Therefore, if the hexaploid race arose originally as an autohexaploid, the pairing relationships of its chromosomes evidently have been altered during the course of development since that time. On the other hand, meiotic pairing indicated considerable homology between chromosomes of the diploid and hexaploid

and between chromosomes of different genomes of the hexaploid. Thus if the hexaploid arose from chromosome doubling in a hybrid of two species, these putative parents must have been closely related and their genomes may have been derived from a common origin. The origin of the hexaploid cannot be postulated, therefore, with the evidence at hand.

The occasional pairing of chromosomes from different genomes, which results in quadrivalent and sexivalent formation, provides a mechanism of exchange of genes between genomes. Such exchanges would be expected only in homologous chromosome segments of sufficient length to permit chiasma formation and in the chromosome regions distal to such homologous segments. For genes located in the homologous regions, segregation ratios might be expected to be intermediate between the typical tetrasomic and the dihybrid ratios. When non-homologous regions occur distal to homologous regions, single crossovers in the homologous segments, followed by distribution of adjacent members of the quadrivalents to the poles in meiosis, might result in the production of non-viable gametic types because of deficiencies and duplications. Thus hexaploids of the chromosomal constitution indicated for *Festuca elatior* var. *arundinacea* might be expected to exhibit some sterility directly attributable to formation and disjunction of the multivalent associations.

Sterility of F_1 hybrids between diploid and hexaploid races of *F. elatior* seriously handicaps efforts to transfer characters from one race to the other. It is possible, however, that more extensive investigations might reveal sufficient fertility to allow for such a transfer. Because of the low incidence of crossing and the sterility of the F_1 hybrids, it is evident that the two races may be handled as separate crops in production of foundation, registered, and certified seed of superior varieties. They may be grown in close proximity without danger of intercrossing.

<center>SUMMARY</center>

In *Festuca elatior* L., plants of the meadow fescue type regularly had $2n = 14$ chromosomes while plants of the tall fescue type, *F. elatior* var. *arundinacea* (Schreb.) Wimm., had $2n = 42$. Both types occurred in natural stands in central Pennsylvania but the diploid was considerably more common.

Meiosis in the diploid was regular while in the hexaploid there were found quadrivalents and, rarely, sexivalents at diakinesis, univalents at metaphase I, laggards at anaphase I and micronuclei in the quartets. Evidence of considerable homology between chromosomes of the diploid and hexaploid races and between chromosomes of different genomes of the hexaploid was provided by the high incidence of pairing at meiosis in F_1 hybrids.

In controlled cross-pollinations between diploid and hexaploid types, few

F_1 seeds were obtained and the hybrid plants were both male and female sterile.

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SECONDARY ROOT STIMULATION IN THE COMMON BEAN, PHASEOLUS VULGARIS L., CAUSED BY THE INSECTICIDES DDT AND COLORADO 9[1]

JESS L. FULTS AND MERLE G. PAYNE[2]

Although much information has been published concerning the use of DDT as an insecticide, very little is known concerning its effects on plants. Two exceptions to this are the work of Appleman and Sears (1) and Payne and Fults (3) who have reported on the depressing effect of DDT on root nodule formation in legumes.

During the progress of these studies the authors observed secondary root stimulation in a few plants which had been grown in soil treated with a high

FIG. 1. Part of the root system of a water control plant of a common bean. Note the number of secondary roots arising from the main laterals. FIG. 2. Part of the root system of a plant of a common bean grown in soil treated with the insecticide DDT, at a high rate of application (127 p.p.m.). Note the increased number of secondary roots on the main laterals.

concentration of DDT. In order to evaluate the significance of this observation, a replicated test of the effects of both DDT and the new insecticide Colorado 9 (2) was made.

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[1] 1-trichloro-2,2-bis-(p-chlorophenyl)ethane (DDT) and 1-trichloro-2,2-bis-(p-bromophenyl)ethane (Colorado 9).

[2] Associate botanist and associate chemist, respectively. Published with the approval of the Director of the Colorado Agricultural Experiment Station as Scientific Series Paper No. 226. The authors are grateful to Andrew G. Clark, Professor of Mathematics, Colorado A. & M. College, for his counsel in connection with the statistical analysis.

MATERIALS AND METHODS

The two chemicals used were made in the laboratories of the Chemistry Section of the Colorado Agricultural Experiment Station as previously described (3). The plant tests were made between January 16 and February 8, 1946, in the greenhouses of the Botany and Plant Pathology Section. Both chemicals were added to the soil (one part sand and three parts clay loam) before planting. This was accomplished by spraying 15 ml. of a chemical emulsion on the surface soil of 6-inch clay pots and allowing it to dry. After drying, the soil from each pot was emptied on a clean sheet of paper, thoroughly mixed, and replaced; then seed was planted. Five pots, each contain-

TABLE 1. *Analysis of variance of the effect of DDT, Colorado 9, and water on the numbers of secondary roots per 6-inch length of lateral root in the common bean.*

Variability due to	D/F	Sum of squares	Mean square (variance)	F value	Required F	
					0.05	0.01
Treatments	2	8,945.90	4,472.95	20.44	3.22	5.15
Within treatments (error)	42	9,191.34	218.84			
Total	44	18,137.34				

Comparison of treatment means by the "t" test

Treatment	DDT	Colorado 9	Water	Difference between means required for significance	
				0.05	0.01
	Secondary roots	Secondary roots	Secondary roots		
Means	65.50	31.33	52.2	10.9	14.6

Comparison	Difference in means	Significance
DDT and Colorado 9	24.27	P > 0.01
DDT and water	13.40	P > 0.05
Colorado 9 and water	20.87	P > 0.01

ing five plants, were used for each of the two chemicals and for the water checks. The amount of chemical used was equivalent to 127 p.p.m. of soil or 103 pounds per acre. At the time of harvest, plants were removed from the pots and the soil was removed from their roots with a fine water spray. Data on numbers of secondary roots were obtained by floating the entire root system on a sheet of glass ruled into 1-inch squares. Since the characters of the root systems of all five plants in each pot treated with a given chemical appeared similar, quantitative data from only one plant per pot were obtained. Within each plant, three lateral roots were selected and the total number of secondary roots arising from a random 6-inch length were counted.

RESULTS AND DISCUSSION

An analysis of variance of the data on the effect of DDT, Colorado 9, and water on the number of secondary roots is presented in table 1. These data show a highly significant F value, and hence the "t" test for minimum significant mean differences was made as shown in the second half of table 1. These results show that treatment with DDT resulted in significantly more secondary roots and with Colorado 9 in significantly fewer than were present in control plants (see figures 1 and 2). None of the treated plants in these tests showed injurious effects in the tops.

Further studies are needed to determine whether or not DDT may be used at high concentrations in the soil to increase the extent of plant root systems under field conditions. Such increased root systems might result in accelerated moisture and nutrient absorption and top growth.

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3. **Payne, Merle G. & Fults, Jess L.** Some effects of 2,4-D, DDT and Colorado 9 on root nodulation in the common bean. [Submitted for publication.]

CROWN GALL DISEASE ON RHUBARB

MICHAEL LEVINE

It is well known that plant tumors can be induced experimentally on many varieties of garden and house plants. The spontaneous development of this disease on garden plants, however, is not of usual occurrence. The sporadic development of crown gall on rhubarb (*Rheum rhaponticum*) grown commercially is of economic interest, as the fate of the crop might be in question. As far as it is known, crown gall on the rhubarb plant has hitherto been unmentioned in the literature. Smith and his colleagues (1911), in their description of the crown gall disease, give a long list of plants tested with *B. tumefaciens*, but fail to mention this species. Inoculations with the crown gall organism made in this laboratory on the petioles of young and mature leaves failed to elicit crown galls. The same response was obtained to treatment with α-naphthalene acetic acid. Necrotic areas were formed at the site of inoculation even though the stimulation of an active growth substance was added.

Recently Professor E. F. Guba sent the writer several specimens of rhubarb roots that consisted of large tumorous masses (fig. 1) apparently attached to or growing from the crown of the plant. Healthy-looking, pinkish-colored buds were also present. Another specimen which followed consisted of a root surrounded by a well formed tumor, as shown in figures 5 and 6. These plant tumors were diagnosed as crown gall; the evidence is pointed out below. The history of the rhubarb culture which yielded these plants appears of interest, for it seems to throw some light on rhubarb culture as well as the crown gall disease. The rhubarb stock known as the "Linnaeus" or "strawberry" variety on which these crown galls appeared, was traced back to 1892. The roots that revealed the crown galls had been growing on the same plot for 15–18 years. In 1945, while the roots were being removed to another farm, an estimated 10 per cent of the stock was found to have these tumorous masses. The purchaser, who saw these galls, refused to take more than half of the crop. The owner, who contended that the galls were of no significance, based his beliefs on the productivity of the stock. This is an interesting point as it relates to the biology of the crown gall on rhubarb and its economic significance.

A number of roots with large crown galls, together with six roots obviously free from the disease, were shipped to this laboratory, where they were set out in a suitable plot in early March, 1946. Figure 7 shows the plot of rhubarb plants fully grown at the end of July. Not a single plant failed to
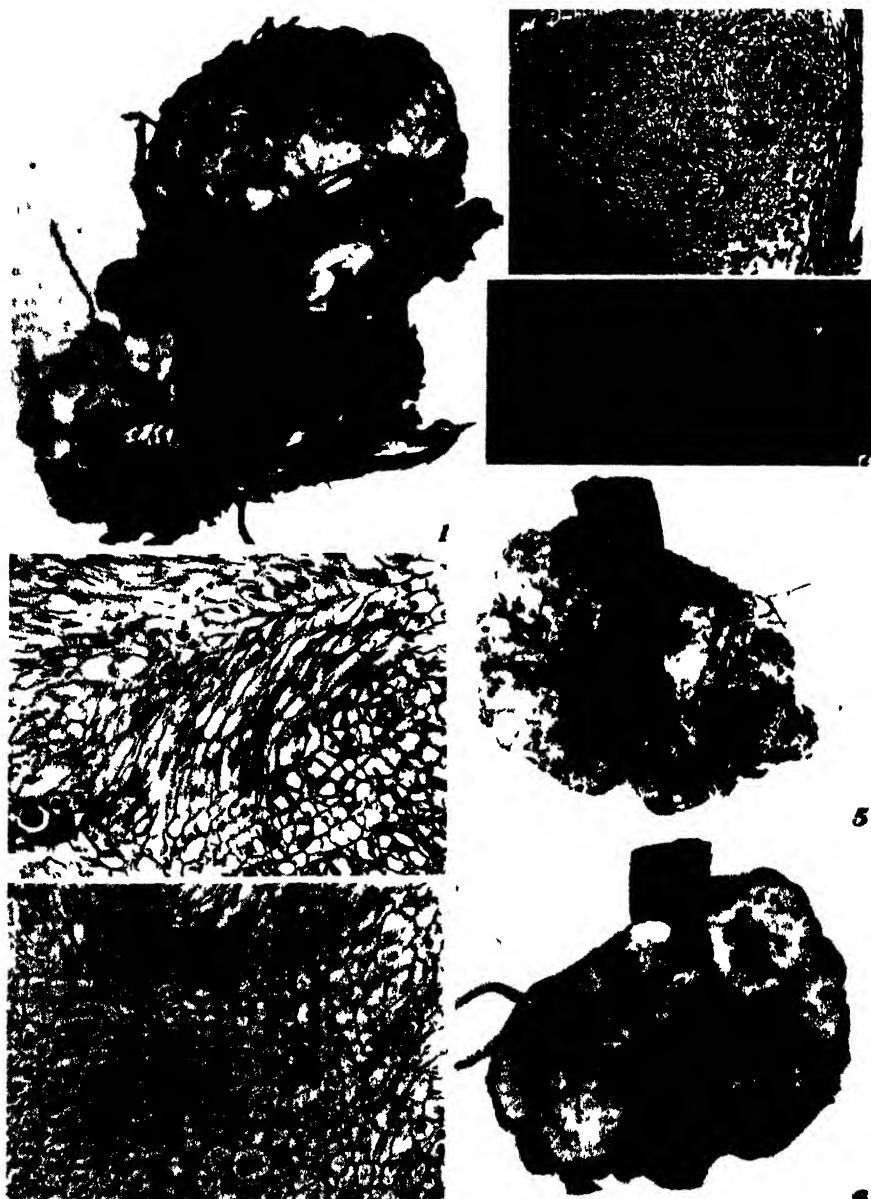
FIG. 1. Root of rhubarb with spontaneous crown gall and developing young leaf buds.
×0.5.   FIG. 2. Section of a nodule of the crown gall shown in figure 1. ×120.   FIGS. 3, 4.
Sections of the gall under higher magnification showing characteristic crown gall tissue.
×240.   FIG. 5. Root of rhubarb with surrounding crown gall. ×0.5.   FIG. 6. Gross section through gall shown in figure 5. ×0.5.   FIG. 7. View of plot in August in which crown-gall-infected and normal roots were planted early in March.

develop a large number of good sturdy leaves with thick succulent petioles. There is no difference between the size and number of leaves of the crown-gall-infected plants and of those without the galls. The fate of the old galls and the development of new galls were under observation. It is clear at this time that the old galls disintegrate. After several plants were dug up early in August, only the decomposed remains of the galls were found. The crowns and roots of the current year's growth appear normal and at this time no crown galls are present. Reinfection of the plant may have been arrested by lack of hoeing or harvesting. The common practice of plucking the leaves may be conducive to infection of the crown.

These observations confirm the studies previously made on experimentally induced crown galls on other garden plants. This overgrowth is not a malignant disease (Levine 1931) in the same category with induced or spontaneous neoplasia of animal or man. The growth appears to be a protective mechanism of limited proliferative capacity. The growth, unlike cancer, matures, grows old, and dies. In successive stages it finally becomes a corky, woody mass of tissue which disintegrates in the soil at the end of the season's growth. In perennial plants the gall above the soil becomes infested with saprophytic growths or dries and breaks off from the branch bearing it. Occasionally on trees and bushes such as the rose reinfection occurs. The old woody gall of the previous season becomes displaced by new growth.

The rhubarb galls of the current year begin to disintegrate in the late fall and the new active roots and crowns may become infected as the result of plucking the leaves. In the spring the leaf buds at the crown develop rapidly and give all appearances of healthy plants. The newly formed galls develop more slowly and do not interfere with the new growth of the leaves. It seems that the commercial product is entirely unaffected. This is borne out by the observations of the grower who only discovered the presence of crown gall disease when the plants were uprooted after twelve years of growth. What effect this disease has on the number of available healthy clones has not been determined. The roots used for propagation purposes could be infected with the crown gall organism at planting time.

The crown gall on the rhubarb plant is a white mass of tissue covered by a heavily mottled brownish-colored coat. The inner structure of a fully formed gall is of a white, firm, fibrous texture (fig. 6). In old galls gathered in November numerous areas of necrosis are seen (fig. 8) on the periphery. At this period many of the galls are in the process of disintegration.

On microscopic examination the fresh gall reveals the characteristic crown gall structure. Figure 2 represents a section through a large smooth nodule on the surface of the main gall shown in figure 1. The parenchymatous cells are small with many crystal-like inclusions. A disoriented array of tracheids and vessels runs through the body of the overgrowth. Under

higher magnification (figs. 3, 4) nucleated protoxylem cells appear in clusters. Few cells were found in division, although the overgrowth was viable, and no evidence of degeneration was present.
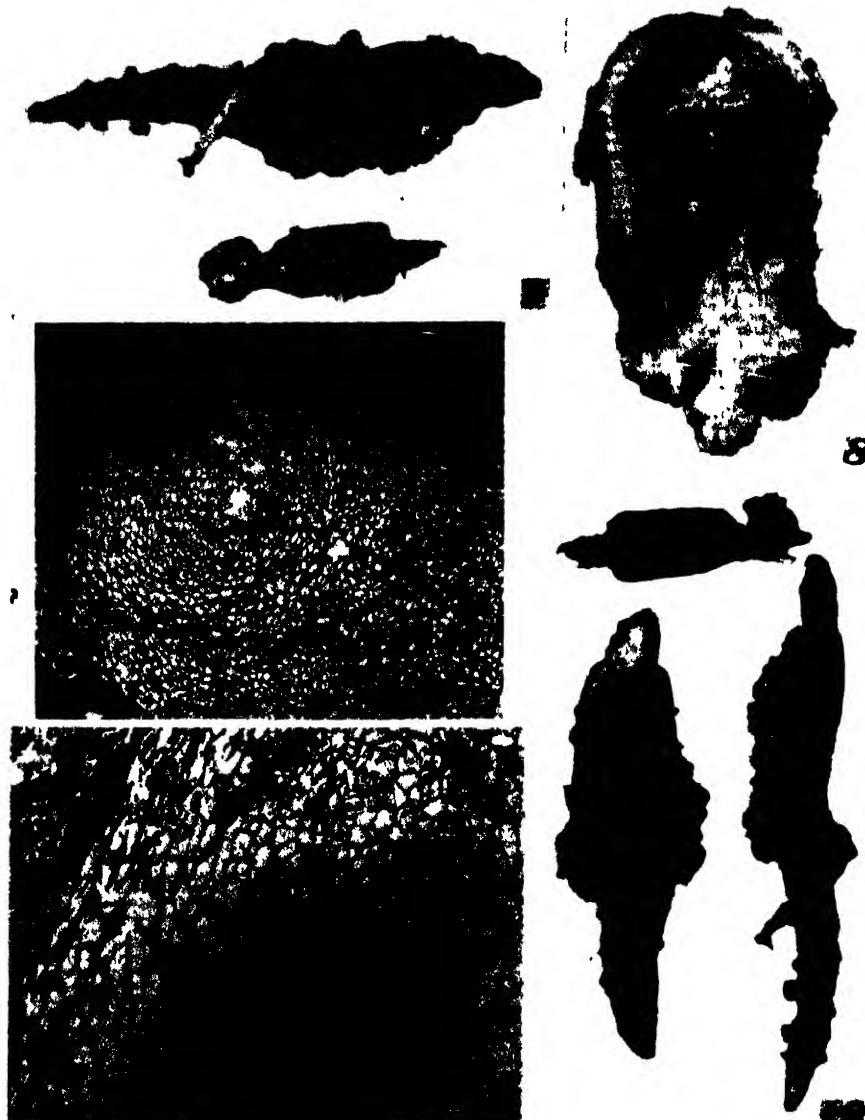
FIG. 8. Gross section through gall showing peripheral necrosis. × 0.5.   FIG. 9. Carrots showing spontaneous crown galls. × 0.5.   FIG. 10. Gross sections of roots shown in figure 9. × 0.5.   FIG. 11. Section through a carrot crown gall. × 60.   FIG. 12. Section under higher magnification showing characteristic crown gall tissue. × 240.

Attempts to isolate the causative organism yielded a rod-shaped schizomycete. Its cultural characteristics were unmistakably similar to those of *B. tumefaciens*. Smears, however, revealed rods with slime in greater abundance than is usually observed on the crown gall organism. The measurements were slightly larger than those of the tumor-producing bacteria. A small number of potted plants were inoculated with the organism but no crown galls larger than well developed scars resulted.

Rhubarb crown gall is another plant tumor that shows all the gross and microscopical morphological characteritstics of crown galls spontaneously or artificially induced. It perpetuates itself from year to year by infecting through the soil injuries induced by propagation. Its effect on the crop of leaves produced in a season is apparently slight as observed over a period of 15–18 years by competent growers.

While this work was in progress, another spontaneously developed gall on the carrot, *Daucus carota*, was brought to this laboratory for identification. The specimens were comparatively small and the root lines were covered with small nodules (figs. 9, 10) not unlike crown galls in appearance. In the photograph of the smaller specimen the tip of the root terminates in a large gall. Microscopic sections of these tumors revealed an abundance of hyperplastic parenchymatous cells (figs. 11, 12) with nodules of scalariform tubes. Little or no hypertrophy was present. The presence of these elements confirmed the diagnosis of crown gall in spite of the fact that some few sections showed nematode invasion of this tissue. Cultural studies of the gall yielded a rod-shaped bacterium which when grown on bean agar had all the characteristics of *B. tumefaciens*. Smears of the organism appeared identical with virulent cultures of *B. tumefaciens*. Inoculations with the organisms, however, produced small crown galls on *Ricinus* grown in the field. Potted plants grown in the laboratory were only slightly reactive to the inoculation with this organism.

SUMMARY

Rhubarb is apparently a new host for the crown gall disease. It occurs on the crown and roots of the plant. The petioles of rhubarb leaves inoculated with the crown gall organism produce no tumors. Spontaneous overgrowths on the garden carrot are typical crown galls produced by a tumor-producing schizomycete. Secondary nematode infection of the crown gall tissue is reported.

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Postscript. On October 17, 1946, all the rhubarb plants were up-rooted for the purpose of ascertaining the number of crown galls, if any, that may have been formed during the growing season. Only one plant produced a large pedunculate crown gall. The tumor was the size of a large grapefruit and was attached to the lower surface of the crown by a short, stubby stalk. The gall was soft and necrotic, and disintegrated on pressure. The rest of the plant was sound and produced a number of leaves equal to that of the other plants. Sixty pounds of marketable petioles and only one gall were produced by the crown-gall-bearing plants set out in March. How infection occurred in this plant is not clear. A moderate number of leaves were plucked throughout the season from all plants. The inoculations made in the petioles produced no tumors.

STUDIES ON PHILIPPINE CHLOROPHYCEAE—
III. THE CODIACEAE

WILLIAM J. GILBERT

The earliest published record of a species of the Codiaceae from the Philippines is *Fucus prolifer* Blanco, which appeared in the first edition of Blanco's *Flora de Filipinas* (1837, p. 838). The description refers to a species of *Halimeda* and may be applied to any one of several species in that area. A few years later Berkeley (1842, p. 157), using the name *Dichonema erectum*, described what he thought to be a fungus but which A. Gepp and E. S. Gepp later showed to be an alga, since known as *Avrainvillea erecta* (Berkeley) A. & E. S. Gepp Following this, papers mentioning the Codiaceae are few and scattered, the more important being Martens (1866), Dickie (1876), and Weber-van Bosse (1913). More recent papers dealing at all with Philippine Chlorophyceae have contained no records of the Codiaceae except for the description of two new species of *Codium* from the South China Sea (Tseng & Gilbert 1942).

Sources of material for this study are those mentioned previously by the writer (1942, 1943) with the addition of two smaller collections. Of these latter one was made in 1937 by Dr José V. Santos at Puerto Princesa, Palawan, and the other by Dr. Gregorio T. Velasquez in the vicinity of Puerto Galera, Mindoro. As in most collections of tropical marine algae, members of the Codiaceae, especially of *Halimeda*, were abundant.

Twenty-three species of Philippine Codiaceae are discussed in the following pages. No attempt has been made to include species which have been reported heretofore from the Philippines but which are not found in the collections studied. These are few, however, and only two have come to the attention of the writer which do not resolve into synonomy with some species on the following pages. One of these is *Halimeda cuneata* Kützing (not *H. cuneata* Hering) reported by Martens (1866, p. 25) and which is now considered to be *Halimeda gracilis* Harvey. The second is the report of *Codium tomentosum* (Hudson) Stackhouse by Dickie (1876, p. 243), now known as *C. dichotomum* (Hudson) S. F. Gray. Geographically both of these species may be expected in this area.

Of the twenty-three species listed eleven appear to be new records for the Philippines, with two genera, *Chlorodesmis* and *Tydemania*, reported for the first time. The new records include *Chlorodesmis comosa, C. Hildebrandtii, C. formosana, Tydemania expeditionis, Udotea javensis, Codium coronatum, C. Geppii, C. contractum, C. intricatum, Halimeda orientalis,* and *H. Monile.*

CHLORODESMIS Bailey & Harvey

CHLORODESMIS COMOSA Bailey & Harvey, 1851, p. 373; A. & E. S. Gepp, 1911, p. 14, *pl. 8, f. 69–73.*

Collections: *Merrill 9161*, Taytay, Palawan, May; *Ebalo A–30*, Punta Mangal, Basilan Island, Jan.; *Bartlett A–91*, near Philippine Desiccated Coconut Factory, Zamboanga, Jan.–Feb.; *Balhani 159*, Lanhil (Little Sibago) Island, Jan.–Feb.; *873*, Saluping Island, due S. of Cabengbeng, Basilan Island, Jan.–Feb.

CHLORODESMIS HILDEBRANDTII A. & E. S. Gepp, 1911, p. 16, 137, *pl. 8, f. 74, 75a, pl. 9, f. 75b;* Tseng, 1936, p. 163, *f. 22.* Collections: *Bartlett 15001, 15033*, Dalupiri Island, Babuyan Group, Oct.–Nov.

The range of diameters of the ascending filaments in the material at hand is somewhat less than the 80–130 μ called for by the original description. A few of the filaments reach a diameter of 115 μ.

CHLORODESMIS FORMOSANA Yamada, 1925, p. 92, *f. 5;* Okamura, 1930, p. 104. Collection: *Bartlett 15034*, Dalupiri Island, Babuyan Group, Oct.–Nov.

AVRAINVILLEA Decaisne

AVRAINVILLEA ERECTA (Berkeley) A. & E. S. Gepp, 1911, p. 29, *pl. 9, f. 84, 85; pl. 10, f. 86–89. Dichonema erectum* Berkeley, 1842, p. 157, *pl. 7, f. 11;* Collections: *Santos 658*, Buenavista, Puerto Princesa, Palawan, April; *Balhani 699*, Kilay (Langasmati) Island, near Langas Island, off the W. coast of Basilan Island, Jan.–Feb.

TYDEMANIA Weber-van Bosse

TYDEMANIA EXPEDITIONIS Weber-van Bosse, 1901, p. 139; 1913, p. 116; A. & E. S. Gepp, 1911, p. 66, *f. 153, 154; Rudicularia penicillata* Heydrich, 1903, p. 97, *f. 1–4.*

Collections: *Bartlett 13988*, Puerto Galera, Mindoro, May; *Velasquez 777*, Paniquian Island, near Puerto Galera, Mindoro, April; *989*, Northwest Channel, Medio Island, near Puerto Galera, Mindoro, April; *1066*, Boaya Point, vicinity of Puerto Galera, Mindoro, April; *Bartlett 16039*, Siasi Island, Sulu Archipelago, Sept.

The known range of *Tydemania expeditionis* has been considerably extended since its discovery in the Siboga material and subsequent description, for it has since been reported from the Indian Ocean, Caroline and Liu-kiu[1] Islands, and now from the Philippines.

UDOTEA Lamouroux

UDOTEA JAVENSIS (Montagne) A. & E. S. Gepp, 1904, p. 363, *pl. 467, f. 1–4;* 1911, p. 110, *pl. 5, f. 36; Rhipidosiphon javensis* Montagne, 1842, p. 14. Collections: *Merrill 9140 p.p.*, Taytay, Palawan, April; *Ebalo A–11a*, Tumakid, Basilan Island, Jan.

UDOTEA ORIENTALIS A. & E. S. Gepp, 1911, p. 119, *f. 1, 4, 47, 48;* Weber-van Bosse, 1913, p. 117; Yamada, 1934, p. 74, *f. 42, 43. Udotea conglutinata* Okamura (not Lamouroux), 1908, 1(9) : 231, *pl. 44, f. 11, 12, pl. 45, f. 8–13.*

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[1] Liu-kiu is the spelling used for that group of islands known also as Riu-kiu, Ryu-kyu, Lu-chu, or Loochoo Islands.

Collections: *Bartlett 14609*, Dalupiri Island, Babuyan Group, July; *15024*, Dalupiri Island, Babuyan Group, Oct.–Nov.; *11145*, Santa Cruz, Zambales Province, Luzon, May; *Merrill 9140 p.p.*, Taytay, Palawan, April.

UDOTEA ARGENTEA Zanardini var. SPUMOSA A. & E. S. Gepp, 1911, p. 126, *pl. 2, f. 15, pl. 3, f. 25a, pl. 7, f. 61, 62;* Weber-van Bosse, 1913, p. 117. Collections: *Bartlett 15587*, small island opposite Culion Harbor, Culion Island, Calamian Group, July; *Merrill 9149*, Taytay, Palawan, April.

UDOTEA FLABELLUM (Ellis & Solander) Lamouroux, 1812, p. 186; *Corallina Flabellum* Ellis & Solander, 1786, p. 124, *pl. 24; U. Flabellum* Howe, 1904, p. 94. Collection: *Bartlett A-199*, Little Santa Cruz Island, opposite Zamboanga, Jan.–Feb.

CODIUM Stackhouse

CODIUM ADHAERENS (Cabrera) C. Agardh, 1822, p. 457; *Agardhia adhaerens* Cabrera (ms.) in Phys. Sällsk. Årsber (quoted from De Toni, 1889, p. 489). Collection: *Bartlett 13788*, Puerta Galera Bay, Puerto Galera, Mindoro, May.

CODIUM CORONATUM Setchell, 1926, p. 82, *pl. 10, f. 2–5, pl. 11, f. 2, 3, pl. 12, f. 1, 5;* Børgesen, 1936, p. 67; Tseng, 1938, p. 148.

Collections: *Velasquez 753*, Balatero Maliit, vicinity of Puerto Galera, Mindoro, April; *825, 1017*, Balete, Varadero Bay, near Puerto Galera, Mindoro, April; *878*, Balatero Malaki, near Puerto Galera, Mindoro, April; *1071*, Ensanada, vicinity of Puerto Galera, Mindoro, April; *Bartlett 13953*, Puerto Galera Bay, Puerto Galera, Mindoro, May; *14016*, Batangas Channel, Puerto Galera, Mindoro, May; *Merrill 9146*, Taytay, Palawan, April; *Santos 656*, Buenavista, Puerto Princesa, Palawan, April; *Balhani 655*, Sibakel Island, Zamboanga Province, Jan.–Feb.; *681*, Tengolan Island (W. of Malusa Bay, Basilan Island), Jan.–Feb.

CODIUM GEPPII Schmidt, 1923, p. 50, *f. 33; C. divaricatum* A. & E. S. Gepp, 1911, p. 145; Weber-van Bosse, 1913, p. 119.

Collections: *Bartlett 16238*, Dalupiri Island, Babuyan Group, July; *Fénix, Bureau of Science no. 13079*, San Fernando, La Union Province, Luzon, Feb.; *Velasquez 1018*, Balete, Varadero Bay, near Puerto Galera, Mindoro, April; *1063*, Boaya Point, vicinity of Puerto Galera, Mindoro, April; *Bartlett 13789, 13997*, Puerto Galera, Mindoro, May.

CODIUM INTRICATUM Okamura, 1913, **3**(4): 74, *pl. 120, f. 9–13;* Schmidt, 1923, p. 55; Yamada, 1934, p. 79; Tseng, 1936, p. 169, *f. 26c.* Collection: *Balhani 590*, Sikeang, S. W. of Punta Matangal, E. coast of Basilan Island, Jan.–Feb.

It is with some hesitation that this specimen is assigned here because the utricles measure but 115–255 µ in diameter and thus do not begin to approach the measurements (770–1150 µ diameter) of the original description. But since the external morphology of the plant as well as the shape of the utricles agree so well with Okamura's original description it is felt that it can best be placed here. Yamada (1934, p. 79) and later Tseng (1936, p. 170) have both referred to this species plants with utricles not much larger than in the material at hand.

CODIUM BARTLETTII Tseng & Gilbert, 1942, p. 291, *f. 1, 2a.*

Collections: *Velasquez 754,* Balatero Maliit, vicinity of Puerto Galera, Mindoro, April; *809,* Hondura Bay, near Puerto Galera, Mindoro, April; *879,* Balatero Malaki, vicinity of Puerto Galera, Mindoro, April; *993,* Paniquian Island, vicinity of Puerto Galera, April; *1046,* Sabang, near Puerto Galera, Mindoro, April; *Bartlett 13954,* Puerto Galera Bay, Puerto Galera, Mindoro, May; *14015,* Batangas Channel, Puerto Galera, Mindoro, May.

CODIUM CONTRACTUM Kjellman, 1897, p. 35, *pl. 2, f. 12, pl. 7, f. 1–3;* Okamura, 1913, **3**(4): 70, *pl. 120, f. 1–8;* Schmidt, 1923, p. 57, *f. 38, 39.* Collection: *Bartlett 14595,* Cavite, Cavite Province, Luzon, July.

CODIUM PAPILLATUM Tseng & Gilbert, 1942, p. 293, *f. 2b–d, 3.* Collections: *Bartlett 14595a,* Cavite, Cavite Province, Luzon, July; *Shaw 1146,* Lamao, Province of Bataan, Luzon, May; *Bartlett 13786,* Puerto Galera Bay, Puerto Galera, Mindoro, May.

CODIUM sp. *Balhani 631,* Panigayan Islands, W. of Isabela de Basilan, Jan.–Feb.

This is a fragmentary specimen, apparently of erect growth, in which the segments are cylindrical, 3–4 mm. broad, and here and there attached to one another by tufts of rhizoidal filaments. The plant is sterile. The utricles are clavate to pyriform, or obovoid, 330–540 μ broad and 710–850 μ long, and have rounded or truncate apices. The size and shape of the utricles agree well with *Codium Mülleri* Kützing, which may be expected in this region, but that species does not appear to have the segments connected by tenacula, nor does the material at hand have the much thickened apical wall characteristic of the utricles of *C. Mülleri.* It seems quite likely that the plant has not been described but it cannot be treated satisfactorily in its fragmentary and sterile condition.

HALIMEDA Lamouroux

HALIMEDA TUNA (Ellis & Solander) Lamouroux f. TYPICA Barton, 1901, p. 13, *pl. 1, f. 1.* This form is characterized by segments that are reniform, discoid, or transversely oblong, the segments reaching 2 cm. broad and 1.2 cm. high.

Collections: *Bartlett 14954,* Currimao, Ilocos Norte Province, Luzon, Oct.; *Williams, R. S.* Daron, Mindanao, March; *Bartlett 16158,* Little Santa Cruz Island, Basilan Strait, Zamboanga, Mindanao, Sept.; *Balhani 309,* Bilang bilang, near Moro Community, Zamboanga Province, Jan.–Feb.; *750,* Boboh, near Punta Matangal, E. point of Basilan Island, Jan.–Feb.

HALIMEDA TUNA f. *platydisca* (Decaisne) Barton, 1901, p. 14, *pl. 1, f. 2; H. platydisca* Decaisne, 1842, p. 102. The segments are larger than in the form above, reaching 3.4 cm. in width and 2.5 cm. in height, and are reniform, discoid, or transversely ovoid.

Collections: *Velasquez 835,* Paniquian Island, vicinity of Puerto Galera, Mindoro, April; *990,* Northwest Channel, Paniquian Island, vicinity of Puerto Galera, Mindoro, April; *864,* east shore of Muelle, vicinity of Puerto Galera, Mindoro, April; *Bartlett 13954, 13991,* Puerto Galera, Mindoro, May; *15585,* small island opposite Culion Harbor, Culion Island, Calamian Group, July; *Ebalo A–7, A–25,* Basilan Island, Jan.; *Balhani 338, 523, 612, 793, 804, 827,* Basilan Island, Jan.–Feb.; *653,* Sibakel Island, Zamboanga

Province, Jan.–Feb.; *698*, Kilay (Langasmati) Island, near Langas Island off the W. coast of Basilan Island, Jan.–Feb.; *817*, Kauluan Island off the S. E. coast of Basilan Island, Jan.–Feb.; *Bartlett 16034*, Siasi Island, Sulu Archipelago, Sept.

HALIMEDA DISCOIDEA Decaisne f. TYPICA Howe ex Børgesen, 1911, p. 134. The segments are discoid, obovate, or somewhat cuneate, in general wider than their length.

Collections: *Bartlett 14618*, Dalupiri Island, Babuyan Group, July; *Velasquez 986*, San Isidio, vicinity of Puerto Galera, Mindoro, April; *Balhani 177*, Pangapuyon Island, Zamboanga Province, Jan.–Feb.; *274*, Sangali, near Bolong, 19 miles N. N. E. of Zamboanga, Jan.–Feb.; *321*, Balas, near Lamitan, N. E. coast of Basilan Island, Jan.–Feb.

HALIMEDA DISCOIDEA f. **subdigitata** Gilbert, f. nov. Planta usque ad 18 cm. alta, articulis inferioribus late cuneatis, superioribus anguste cuneatis vel digitatis.

TYPUS: *H. H. Bartlett 16036*, ex Siasi Island, Sulu Archipelago, Insulis Philippinis, 15 IX 1935, in Herb. Univ. Michiganensi.

This form of *Halimeda discoidea* reaches 18 cm. in height, and differs from the typical form by having the lower segments broadly cuneate, and those above narrowly cuneate to finger-like. From the top of some of the broader segments originate 3–8 segments digitately.

Collections· *Velasquez 824*, Varadero Bay, vicinity of Puerto Galera, Mindoro, April; *1023*, San Teodora Municipality, near Puerto Galera, Mindoro, April; *Merrill 9147*, Taytay, Palawan, April; *Bartlett A–89*, near Philippine Desiccated Coconut Factory, Zamboanga, Jan.–Feb.; *A–197*, Little Santa Cruz Island, opposite Zamboanga, Jan.–Feb.; *Balhani 124*, Sibago Island (at E. entrance to Strait of Basilan), Jan.–Feb.; *141*, Tabtabon (Tictavon) Island, S. E. of Zamboanga, Jan.–Feb.; *157*, Lanhil (Little Sibago) Island, Jan.–Feb.; *310*, Bilang bilang, near Moro Community, Zamboanga Province, Jan.–Feb.; *426*, Labuan, 20 miles N. W. of Zamboanga, Jan.–Feb.; *470*, Bavara, Pangapuyan (Pangapuan) Island, Zamboanga Province, Jan.–Feb.; *872*, Saluping (Salipin) Island, due S. of Cabengbeng, Basilan Island, Jan.–Feb.; *Bartlett 16036*, TYPE, Siasi Island, Sulu Archipelago, Sept.

Because the external morphology of this and the following form so strongly suggests *Halimeda cuneata* Hering forms *digitata* Barton and *typica* Barton some explanation is required. For some time many of the plants listed above under *H. discoidea* were considered to be representatives of *H. cuneata*. The writer was influenced primarily by the similarity in form between these plants and the figures presented by Barton (1901, p. 16, *pl. 2, f. 7, 9*) for the aforementioned forms of *H. cuneata*. The relative ease with which the fused nodal filaments were separable upon decalcification and the somewhat greater diameter of the cortical utricles than is usual in *H. cuneata* suggested doubt concerning the validity of the determinations. A closer microscopical examination then revealed that every specimen on hand possessed the large, bullate, subcortical utricles which are characteristic only of *H. discoidea* to which Howe (1907, p. 496) first called attention. Some of the subcortical utricles reach 200 µ in diameter and they always appear much larger than the 6–14 cortical utricles borne on them. The large size of the subcortical utricles, the relative ease of separation of the fused nodal filaments, the relatively large subquadrate cortical utricles, and the absence of

a cushion-like base to the segments indicated that the plants were specimens of *Halimeda discoidea* and not *H. cuneata*.[2]

Inasmuch as Barton (1901) made no mention of subcortical utricles in her outstanding monograph of the genus *Halimeda*, there is a real possibility that all or part of the plants she designated as *H. cuneata* f. *digitata* may be *H. discoidea* f. *subdigitata*. Likewise, part of the plants she considered *H. cuneata* f. *typica* may be the following form of *H. discoidea*.

HALIMEDA DISCOIDEA f. **intermedia** Gilbert, f. nov. Planta usque ad 7.5 cm. alta, articulis regulariter cuneatis, rarum ovatis, inferioribus 7.5 mm. latis, 1.0 cm. longis, superioribus minoribus.

TYPUS: *H. H. Bartlett 15002*, ex Dalupiri Island, Babuyan Group, Insulis Philippinensibus, 31 X–5 XI, 1935, in Herb. Univ. Michiganensi.

This form reaches a height of 7.5 cm., with the segments nearly all typically cuneate, becoming smaller towards the tips of the branches. The shape of the cuneate segments is quite intermediate between the discoid segments of f. *typica* and the narrowly cuneate to digitate shape of most of the segments of f. *subdigitata*. The segments are generally not as broad as long.

Collections: *Bartlett 15002*, TYPE, *15003*, *15005*, *15010*, all from Dalupiri Island, Babuyan Group, 31 Oct.–5 Nov.; *A–11*, Lagoon of Big Santa Cruz Island, opposite Zamboanga, Jan.–Feb.; *A–90*, near Philippine Desiccated Coconut Factory, Zamboanga, Jan.–Feb.; *Balhani 66*, Atong atong, near Malamaui, N. coast of Basilan Island, Jan.–Feb.; *214, Baliwasan* (Baliuasan), N. W. of Zamboanga, Jan.–Feb.; *386*, Mameaan (Mameahan), 15 miles N. E. of Zamboanga, Jan.–Feb.

HALIMEDA MACROLOBA Decaisne, 1842, p. 118; Harvey, 1863, Phycol. Austr. **5**: *pl. 267;* Barton, 1901, p. 24, *pl. 3, f. 33–38;* Okamura, 1915, **3**(10): *pl. 149, f. 1–8.*

Collections: *Bartlett 15415*, Basco, Batan Island, Batanes Province, July; *14956*, Currimao, Ilocos Norte Province, Luzon, Oct.; *Merrill, Species Blancoanae No. 993*, coral reefs, Puro, San Fernando, Union Province, Luzon, Oct. (as *Halimeda* sp.); *Yates, Bureau of Science 25829*, Basud, Tayabas Province, Luzon, Dec.; *Villaflores 44*, Lubang, Mindoro Province, Sept.; *Bartlett 13932, 13961, 14059*, vicinity of Puerto Galera, Mindoro, May; *Velasquez 733, 744, 761, 863, 882, 991, 1016*, near Puerto Galera, Mindoro, March and April; *Bartlett 15574*, small island opposite Culion Harbor, Culion Island, Calamian Group, July; *Merrill 9151*, Taytay, Palawan, April; *Fénix, Bureau of Science no. 15815*, Zamboanga, Mindanao, Aug.; *Ebalo A–8*, Tumakid, Basilan Island, Jan.; *Balhani 305*, Bilang-bilang, Zamboanga Province, Jan.–Feb.; *719, 751, 773*, Basilan Island, Jan.–Feb; *786*, Baluk-baluk Island, W. of Basilan Island, E. of Pilas Island, Jan.–Feb.; *J. B. Steere*, Balabac Island (no collection number or date.)

Halimeda orientalis Gilbert, sp. nov. Figure 1. Planta (in fluido) usque ad 6.0 cm. alta, multi-ramosa in parte inferiore; basi rhizoideo inconspicue;

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[2] To the writer it now appears rather doubtful that *Halimeda cuneata* Hering is as widely distributed as formerly indicated. It has been reported from a number of places such as Japan, Liu-kiu Islands, China Sea, Formosa, Malay Archipelago, and New Guinea, as well as from Natal Bay in South Africa whence it was first described. The writer has not seen material from any locality other than South Africa which proved to be *H. cuneata* although several sets have been studied that were published and distributed as that species from regions other than South Africa, as, for instance, Yamada's Liu-kiu plant and Higashi's material from Hamajima, Shima. Instead the plants usually prove to be *Halimeda discoidea* Decaisne.

articulis circa 9 mm. latis, 6 mm. longis, minus quam 1.0 mm. crassis, interdum inconspicue nervatis; inferioribus crassis, ad basin subcuneatis vel sub-truncatis, margine integerrimis aut conspicue lobatis, interdum conjunctis; superioribus complanatis, orbiculatis vel reniformibus, margine integerrimis aut crenulatis. Filamenta centralia haud cohaerentia, membrana solum ad apicem articulorum incrassata. Utriculi corticis non cohaerentes, pyriformes vel subclavati, 26–41 μ lati, 36–72 μ longi, modice calce incrustati.

TYPUS: *Liborio E. Ebalo A-20,* ex Tumakid, Basilan Island, Insulis Philippinis, January, 1941, in Herb. Univ. Michiganensi.

This new species of *Halimeda* reaches a height of 6.0 cm., but the usual height is about 5 cm. Branching near the base is abundant and in more than one plane so that a thick tuft of branches is formed (somewhat as in *H.*
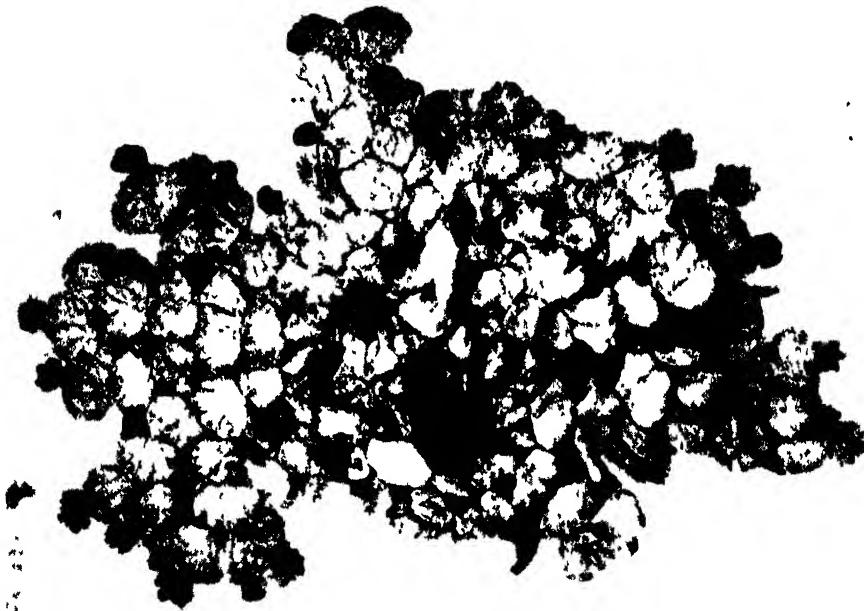
FIG. 1. *Halimeda orientalis* Gilbert, sp. nov. × 1.5.

Opuntia). Above the branches are fewer and more or less in one plane. The rhizoidal base is indistinct. Near the base of the plant the segments are irregularly cuneate or truncate, are thickened, and may be either entire or three-lobed. In some instances the basal segments are fused together and are many lobed. Above the segments are clearly flattened, discoid to somewhat reniform, with an entire or crenulate upper margin. Occasionally cuneate and truncate bases are found in the terminal segments. The normal segments reach a width of 9 mm. or less, which may be exceeded in the occasional fused segments at the base of the plant, and they are 6 mm. or less in height. In thickness the segments are less than 1 mm. Some of the segments are indistinctly ribbed. The central filaments at the apex of the segments have thickened walls but are entirely free. They are loose in the upper nodes, crowded

in the lower nodes, without pits, and easily separable upon teasing. The cortical utricles are pyriform to subclavate and are not coherent. Being free they are held together only by the deposition of lime and thus readily fall apart upon decalcification. The average maximum diameter of the cortical utricles is 37 μ, ranging from 26–41 μ. In length the cortical utricles are 36–72 μ. The subcortical utricles are equal to or less than the diameter of the of the peripheral utricles. Calcification is only moderate, and when dry the segments are whitish to greyish green, darker above.[3] Collection: *Ebalo A-20,* TYPE, Tumakid, Basilan Island, January.

This new *Halimeda* is characterized by the noncoherence of both cortical utricles and central filaments and by branching in more than one plane. Regarding coherence of utricles two other species of Halimeda are recognized which have no contact between the cortical utricles. One, *Halimeda macroloba* Decaisne, differs from *H. orientalis* by its more massive size, its degree of calcification, the greater length of its cortical utricles, and especially by the strong coherence and intercommunication of its central filaments at the nodes. The other species with free cortical utricles is *H. macrophysa* Askenasy but it is distinct from *H. orientalis* since in the former the central filaments are actually fused in twos and threes at the nodes. The cortical utricles are also much larger. Neither *H. macroloba* nor *H. macrophysa* branch in more than one plane.

Considering next the characteristic of free central filaments attention is drawn to the plants which Barton (1901, p. 27, *pl. 4, f. 42, 47*) included under *Halimedia incrassata* (Ellis & Solander) Lamonroux f. *ovata* (J. Agardh) Barton. These not only somewhat resemble the new species in external form but Barton noted that the central filaments at the apex of the segments are entirely free or connected by small and few pits. Barton also called attention to the occasional absence of pits in other forms of *H. incrassata* but spoke of free central filaments only in the plants which were placed under f. *ovata.* The similarity between the free central filaments in *H. orientalis* and Barton's figures of the same for *H. incrassata* f. *ovata* is striking. One is thus led to consider whether the plants Barton included under *H. incrassata* f. *ovata* might not actually be (in part, at least) the new species. Since Barton's material has not been seen in the present study this question can not be answered with finality now. It is interesting to note, however, that Barton did not have access to Agardh's type material of *H. incrassata* var. *ovata.* On the other hand Howe (1907, p. 503), who did make a detailed study of the type material of *H. incrassata* var. *ovata,* for comparison with *H. simulans,* made no mention of either free central filaments or noncoherence of cortical utricles. It seems most certain that if either central filaments or corticle utricles had been free it would have been noted by Howe for *H. simulans* has strongly coherent central filaments at the segment apices, as well as coherent utricles.

With reference to the manner of branching *Halimeda Opuntia* is the only other species in which the branching is in more than one plane but it can be readily separated from *H. orientalis* by its coherent utricles, its characteristic manner of incomplete fusion of central filaments at the apex of the segments, and by its distinctly ribbed segments.

Halimeda orientalis stands in the section of the genus in which the central filaments are not fused, probably closest to *H. macroloba* on the basis of non-coherence of cortical utricles and next to *H. tridens* (*H. incrasata*), especially those forms in which the pits between the cohering central filaments are few or wanting.

HALIMEDA OPUNTIA (L.) Lamouroux f. TYPICA Barton, 1901, p. 20, *pl. 2, f. 19.* The segments are distinctly ribbed, giving the upper margin a lobed appearance; the lower margins of the segments are about at right angles with the midribs, the joints not overlapping.

Collections: *Albatross Expedition 14; McGregor 47*, Calayan Island, Cagayan Province, (1903); *Bartlett 14619*, July, *15006*, Nov., Dalupiri Island, Babuyan Group; *Yates, Bureau of Science no. 25830 pp.*, Basiad, Province of Tayabas, Luzon, Dec.; *Villaflores 23*, Lubang Island, Mindoro Province, Sept.; *Bartlett 13933*, Puerto Galera, Mindoro, May; *Velasquez 761, 793, 843, 883, 1013, 1067*, all in April, vicinity of Puerto Galera, Mindoro; *Bartlett 16182*, Liloan Beach, E. coast of Cebu Island, Sept.; *Santos 649, 700*, vicinity of Puerto Princesa, Palawan, April; *Bartlett A-198*, Little Santa Cruz Island, opposite Zamboanga, Mindanao, Jan.–Feb.; *Ebalo A-13, A-21, A-32*, Basilan Island, Jan.–Feb.; *Balhani 67, 111, 123, 178, 235, 275, 290, 325, 352, 371, 387, 407, 408, 425, 440, 454, 483, 497, 508, 525, 536, 545, 566, 589, 603, 654, 683, 718, 737, 738, 752, 774, 818, 828, 829*, all from the vicinity of Zamboanga, Mindanao, and Basilan Island, Jan.–Feb.; *Bartlett 16121, 16122*, N. Ubian Island, Sulu Archipelago, Sept.

In the specimens above there is evident variation toward other forms which involves either the whole specimen or merely a part of it, often the latter. Portions of *Bartlett A-198* and *16122* come close to f. *triloba* while *Bartlett 16182* approaches f. *cordata.* Likewise *Balhani 526, 818*, and *829* approach f. *hederacea* with a segment here and there having the typical "ivy-leaf" shape of that form.

HALIMEDA OPUNTIA f. CORDATA (J. Agardh) Barton, 1901, p. 20, *pl. 2, f. 21; H. cordata* J. Agardh, 1886, p. 83. The segments are rounded above and at their bases cordate with the auriculate projections overlapping the segments below. Collection: *Merrill 9141*, Taytay, Palawan, April.

HALIMEDA OPUNTIA f. TRILOBA (Decaisne) Barton, 1901, p. 20, *pl. 2, f. 20; H. triloba* Decaisne, 1842, p. 102. In this form the segments are distinctly trilobed, especially in the lower portion of the plant, rather thick, and with evident ribs.

Collections: *Albatross Expedition no. 29; Bartlett 15883, 15589*, from small island opposite Culion Harbor, Culion Island, Calamian Group, July; *Velasquez 994*, Paniquian Island, vicinity of Puerto Galera, Mindoro, April; *Bartlett 16198*, Cebu Island, Sept.; *16146*, Little Santa Cruz Island, Basilan Strait, Zamboanga, Mindanao, Sept.; *Balhani 257*, Calarian (Kaladian) near Army Post, Zamboanga, Jan.–Feb.; *613, 792*, Basilan Island, Jan.–Feb.; *Bartlett 16035*, Siasi Island, Sulu Archipelago, Sept.

There is considerable variation among the specimens representing this form. *Bartlett 15589*, for instance, has the upper parts approaching f. *typica*

while the lower parts are unmistakably like f. *triloba*. Specimens of *Bartlett 16035* are in good fruiting condition and it is interesting to note that the tufts of sporangia originate from the tips of the lobes instead of along the entire upper margin.

HALIMEDA OPUNTIA f. RENCHII (Hauck) Barton, 1901, p. 21, *pl. 2, f. 22, 22a; H. Renchii* Hauck, 1886, p. 167. In this form the segments are comparatively small, round, cuneate or ovate, and with indistinct ribs; the branches more or less radiate from a common center. Collections: *Bartlett 15007, 15008*, Dalupiri Island, Babuyan Group, Nov. Approaching in part f. *intermedia* Yamada, *Bartlett 15008* appears to represent a transition between the two forms.

HALIMEDA OPUNTIA f. INTERMEDIA Yamada, 1934, p. 81, *f. 50, 51.* The segments are round or ovate, or sometimes with slightly cordate bases, and in appearance are much like the segments of f. *Renschii* but larger, varying in size and reaching 8 mm. in width and 6 mm. in height. Collection: *Santos 118*, Wawa, Nasugbu, Batangas Province, July. This specimen is fruiting and the tufts of sporangia are quite evenly distributed along the entire upper margin of the segments.

HALIMEDA OPUNTIA f. HEDERACEA Barton, 1901, p. 21, *pl. 3, f. 23.* The segments are usually large, trilobed, having the appearance of an ivy leaf, and with distinct ribs.

Collections: *Yates, Bureau of Science 25830 pp.*, Basiad Tayabas Province, Luzon, Dec.; *Ebalo A-33*, Punta Mangal, Basilan Island, Jan.; *Balhani 256*, Calarian (Kaladian) near Army Post, Zamboanga, Jan.–Feb.; *614*, Liksapi, near Bagbagon, Basilan Island, Jan.–Feb.; *Albatross Expedition 14a.*

The segments are nearly always larger than in other forms of *Halimeda Opuntia*, reaching a width of 18 mm. and height of 13 mm. or less. The surface of the segments is often shiny. Seldom do all of the segments exhibit the ivy-leaf shape as one might suppose from Barton's descriptions and figure, but in *Ebalo A-33* most of the segments are of the typical shape. In *Balhani 256* many of the segments have a cordate base, thus approaching *H. Opuntia* f. *cordata*.

HALIMEDA MONILE (Ellis & Solander) Lamouroux, 1812, p. 186; *Corallina Monile* Ellis & Solander, 1786, p. 110, *pl. 20, f. c; H. incrassata* f. *monlis* Barton, 1901, p. 27, *pl. 4, f. 40.* Collection: *Bartlett 15578*, small island opposite Culion Harbor, Culion Island, Calamian Group, July.

HALIMEDA TRIDENS (Ellis & Solander) Lamouroux f. TYPICA (Barton) Collins, 1909, p. 318; *H. incrassata* (Ellis & Solander) Lamouroux f. *typica* Barton, 1901, p. 27, *pl. 4, f. 39.* In this form the lower segments are cylindrical or somewhat compressed, sometimes fused to form a fan-shaped base; the upper segments are distinctly compressed, entire or crenulate, or at times trilobed.

Collections: *Yates, Bureau of Science no. 25831*, Basiad, Province of Tayabas, Luzon, Dec.; *Ebalo A-12*, Tumakid, Basilan Island, Jan.; *Barlett A-88*, near Philippine Desiccated Coconut Factory, Zamboanga, Jan.–Feb.; *Balhani 158*, Lanhil (Little Sibago)

Island (at E. entrance to Strait of Basilan), Jan.–Feb.; *427*, Labuan, 20 miles N. W. of Zamboanga, Jan.–Feb.; *524, 604*, Basilan Island, Jan.–Feb.; *Bartlett 16130* and *16132*, North Ubian Island, Sulu Archipelago, Sept.; *16033* and *16037*, S. of Siasi, Siasi Island, Sulu Archipelago, Sept.

HALIMEDA TRIDENS f. **Lamourouxii** (J. Agardh) Gilbert, comb. nov. *Halimedia incrassata* var. *Lamourouxii* J. Agardh, 1886, p. 86. The lower segments are compressed and broadly cuneate, sometimes lobed; the upper segments are thin, cuneate to discoid, or reniform, with a crenulate or lobed margin; the segments are large and heavily calcified. Collections: *Bartlett 15584*, small island opposite Culion Harbor, Culion Island, Calamian Group, July; *A-196*, Little Santa Cruz Island, opposite Zamboanga, Jan.–Feb.

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ON THE GENUS MITRASTEMON

EIZI MATUDA

INTRODUCTION

In March, 1934, while on an expedition to Mt. Ovando, which is in the municipality of Escuintla, Soconusco district, state of Chiapas, southern Mexico, and while in the so-called *Quercus* vegetational zone at an altitude of 1700 meters, I unexpectedly found a parasitic plant which I instantly. recognized as a species of *Mitrastemon*. The existence of this genus on the American continent is most surprising. For a moment it was difficult to believe my eyes, but since I collected this curious and interesting plant, in full bloom, with my own hands, there could be no doubt. My collection of the plant, both in flower and in fruit, was subsequently preserved in the herbarium of my plantation "La Esperanza," near Escuintla. Two years later— in April, 1936—when I again had the opportunity of botanizing on Mt. Ovando, I found the same parasite in abundance at the same locality. Mt. Ovando is a branch of the Sierra Madre of Chiapas which pushes its way to the so-called valley of Soconusco and has an altitude of 2300 meters.

Because of the fact that at that time I lacked any literature relative to these plants, my collection was sent to and examined by my friend, Dr. Yoshimatsu Yamamoto, distinguished taxonomist of the Taihoku Imperial University of Taiwan, Japan. Dr Yamamoto, after completing his study of the material, proposed for the plant (1) the name *Mitrastemon Matudai* Yamamoto. Upon receipt of reprints of the original description, I distributed these, along with herbarium specimens of the plant, to all the principal herbaria of Mexico and the United States, but professional botanists there apparently gave the discovery very little attention.

In Europe, however, some excitement was caused by this discovery. Dr. Ludwig Diels, late director of the *Botanisches Museum und Garten* at Berlin, wrote to me asking for certain morphological details and for some specimens of the parasite. For the scientific world this was not only a new species, but constituted the record of a new genus and family for the continental American flora. Yamamoto's original description was drawn from a dry herbarium specimen, and should be corrected as follows from observations made on fresh material (figs. 1–9).

Planta parasitica ad radices horizontales *Querci boqueronae,* in vivo lactea nitidaque, in statu exsiccato fusco-nigricans nitidaque, 4–12 cm. longa, cum squamis imbricatis 3–3.5 cm. diam. Receptaculum basale cupuliforme crassum, ca. 1.5 cm. longum, ca. 2.5 cm. diam., plus minusve compressum furvum non nitidum (in vivo), nigro-fuscens non nitidum (in statu exsic-

cato) irregulariter tuberculatum, ad oram irregulariter lobatum, lobis saepe 4, margine rotundatis saepe 1–2-lobulatis. Caulis cylindricus, simplex, 1 cm. diam., erectus, 3–8 cm. longus, squamatus. Squamae imbricatim oppositae, vulgo 7-seriatim dispositae, glabrae nitidaeque in vivo lacteae ad apicem minute ferruginoso-nigricantes, in statu exsiccato ad faciem badiae vel fulvo-rubescentes, inaequilongae; squamae inferiores minores, apicem versus gradatim majores; squamae basales primariae 2 inter se oppositae late ovatae vel rotundatae 1.3 cm. longae, 1.5 cm. latae, ad apicem obtusissimae vel rotundatae intus concavae extus convexae et leviter longitudinaliter rugosae; squamae secundariae 2 inter se suboppositae et cum primariae alternae rotundatae 1.8 cm. longae, 2 cm. latae, ad apicem obtusissimae intus valde concavae, extus convexae; squamae mediae 2 inter se oppositae deltoideae vel late triangulares 2.8 cm. longae, ca. 2.5 cm. latae, ad apicem obtusae, intus
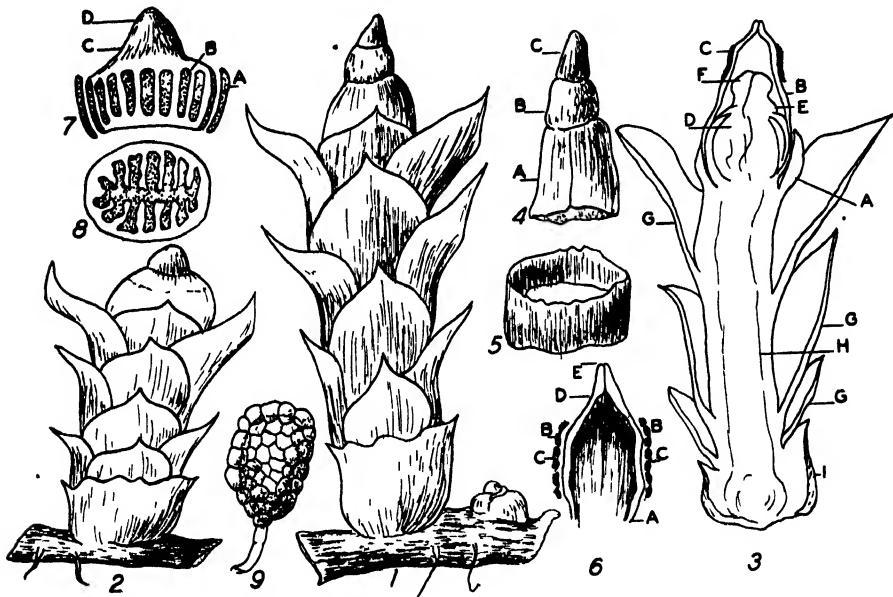
FIGS. 1–9. *Mitrastemon Matudai* Yam. Drawn from fresh material. Figs. 1–5 reduced to 0.9 natural size. FIG. 1. Plant in bloom, with mitriform cap of stamens. FIG. 2. Dwarfed plant, in fruit. FIG. 3. Longitudinal section of whole plant. A, perianth; B, mitriform cap of stamens; C, anther; D, ovary; E, style; F, stigma; G, scale; H, stalk; I, volva. FIG. 4. Stamen. A, connate filament; B, anther; C, point of anther belt. FIG. 5. Perianth. FIG. 6. Longitudinal section of anther belt. A, anther belt; B, pollen sac; C, mucous; D, point of the belt; E, slit. FIG. 7. Mature ovary in longitudinal section. A, perianth; B, ovules; C, conical point. FIG. 8. The same in cross section. FIG. 9. Ovule.

valde concavae extus valde convexae; squamae superiores deltoideae 2 inter se oppositae 2 cm. longae, 2.2 cm. latae, ad apicem obtusae extus convexae, intus concavae et superne valde involutae falciformiter inflexae. Perianthium membranaceum, gamophyllum, breviter cylindricum vel circuliforme, 6 cm. longum, 1.5–1.7 cm. diam., extus longitudinaliter paralleliterque rugosum, in statu exsiccato badium, sed in vivo lacteum, ad oram irregulariter undu-

latum. Androecium hypogynum, calyptriforme, ad apicem obtusum; tubo filamentorum connatorum 2.8 cm. longo, 1.4 cm. diam. glaberrimo lacteo·in vivo, extus longitudinaliter paralleliterque striato in statu exsiccato; tubo antherarum 8 mm. longo, 12 mm. in diam.; tubus androecii ultra antheras productus pyramidalis vel conicus, 3 mm. longus, ad basim 5 mm. diam., longitudinaliter 3-seriatim striatus, seriebus 4 linearibus in statu exsiccato. Fructus solitarius terminalis, erectus sessilis baccatus teres, sed ad faciem longitudinaliter plus minusve sulcato-striatus, cupuliformis vel breve cylindricus et valde compressus, 1 cm. longus, 1.5 cm. diam., unilocularis; placentis 15 crassiusculis lamellatis, stylo brevissimo 2 mm. longo, stigmate conico 7 mm. longo et 7 mm. diam. Semina minima, plurima, obovata, stipitata, ad faciem reticulata.

HISTORY OF THE GENUS

The genus *Mitrastemon* was established in 1909 by Dr. Tomitaro Makino (2), based on a single parasitic species, the type specimen being flowering but of very peculiar character, collected in Koti prefecture, Sikoku, on the southern island of Nippon proper, by Prof. H. Yamamoto of the Normal College of that prefecture. However, this parasite had been widely known to the natives of Kagosima prefecture on Kyusyu—another southern island of Nippon proper—under the name of "Hanasii no Titi," and had actually been recorded in literature by A. Tasiro more than thirty years before Makino's description appeared. Dr. Makino, after further study of more and perfect material of the plant, published another account of it in 1911 and made the proposal entitled "Serie Mitrastemonales, nov.; Fam. Mitrastemonaceae, nov." (3). This marked a new epoch in the history of plant taxonomy in Japan.

In the same year at Mt. Muto in Taiwan (Formosa), then a southern territory of Japan, specimens of another parasite were collected by Eng. T. Kawakami and S. Sasaki. In 1912 Dr. Bunzo Hayata, the well-known systematic botanist of the Tokyo Imperial University, named this new species *Mitrastemon Kawa-Sasakii* Hayata (4). In 1913 Hayata published his · opinion relative to the systematic position of *Mitrastemon*, assigning it to the family Rafflesiaceae in his *Icones plantarum formosanarum* (5). This disposition was adopted by Engler in *Die natürlichen Pflanzenfamilien* (6), and also in Engler & Gilg's *Syllabus der Pflanzenfamilien*," edition 8 (7).

Meanwhile, in Taiwan another new parasitic plant was discovered which was identified by Dr. Y. Yamamoto as a third species, *Mitrastemon Kanehirai* Yam. (8). While occupied in botanical research in southern Taiwan, the writer also collected *M. Kawa-Sasakii* on Mt. Minami-daibu in 1919. In 1929 Dr. Jechens collected a parasite at Brentagi in northern Sumatra, Netherlands East Indies, and this was determined by Dr. Hayata as *M. Kawa-Sasakii* (9).

OBSERVATIONS

The general appearance of these parasites will be understood from the description given above of *M. Matudai*. All the species have approximately the same aspect. The plants are beautiful when alive, resembling wax art work with their shiny lacteous surface. They grow on the horizontal roots of members of the family *Fagaceae*. I have observed that the height of the plant seems to depend on the depth of the host root. Usually there are four upper scale-leaves on the surface of the ground. The root may be from 4 to 10 cm. underground, never deeper. It is a notable fact that in any one region the parasite utilizes as host the roots of only a single species. *M. Matudai* grows only on the roots of *Quercus boqueronae*, on Mt. Ovando in southern Mexico, as far as I am aware. *M. Yamamotoi* Mak. grows on *Shiia Sieboldi* Mak. in Koti, Sikoku, and on *S. cuspidata* Mak. in Kagosima, Kyusyu. *M. Kawa-Sasakii* Hay. grows on *Quercus glauca* Thunb. on Mt. Muto, Taiwan, on *Lithocarpus Kawakamii* Hay. on Mt. Reito, Taiwan, on *Symaedrys brevicaudata* Koidz. on Mt. Roppei, Taiwan, and on *Quercus spicata* Sm. in Sumatra. *M. Kanehirai* Yam. grows on *Castanopsis Kawakamii* Hay. in Rengeti and on *C. taiwaniana* Hay. in another locality on Taiwan.

Although this parasitic genus has a rather wide distribution from the southern islands of Japan proper down through Okinawa, Taiwan, and as far south as Sumatra, below the equator, the climatography of each locality has a definite similarity. For instance, at the northern limit of Koti prefecture we have:

Month	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Average
Temperature	5.4	6.1	9.5	14.7	18.1	21.6	25.2	26.1	23.1	*17.8*	*12.8*	7.2	15.6 C.
Rainfall	66	103	87	284	282	345	326	307	420	*216*	*114*	78	227 mm.

In Koti prefecture the parasite has been collected in October and November, as is indicated by the italic in the table above.

In Kagosima prefecture, as an example of the middle zone of distribution, the following climatic conditions are noted:

Month	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Average
Temperature	10	10	14	14	18	22	28	28	26	22	*18*	*12*	20 C.
Rainfall	100	100	150	150	200	400	250	150	250	200	*100*	*50*	200 mm.

Here, as indicated by the italic, it has been collected in November and December, during the dry season, with the temperature ranging from 12° to 18° C.

On Mt. Muto, in southern Taiwan, in the *Fagaceae* zone, where the plant is found at an altitude of approximately 1400 meters, we observe the following conditions:

Month	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Average
Temperature	*11.4*	*11.4*	11.4	13.4	15.4	19.4	19.4	19.4	19.4	17.4	13.4	11.4	15.4 C.
Rainfall	*35*	*75*	75	75	225	450	550	800	450	175	125	35	275 mm.

Here the italic indicates that the plant grows in January and February, during the dry season, with the temperature at about 11.4° C.

In Sumatra, according to information given by Jochems, at Brastegi, at an altitude of 1500 meters, we have the following conditions:

Month	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Average
Temperature	16.0	16.9	17.7	17.4	18.1	17.5	17.7	18.1	17.6	17.3	17.5	17.5	17.4 C.
Rainfall	206	98	159	219	115	59	109	151	132	175	221	252	157.8 mm.

In Brastegi, where a temperature of 16° to 18° C obtains all through the year, *M. Kawa-Sasakii* grows all the year round.

Lastly, in Mexico, on Mt. Ovando the parasite grows only from February to April, during the dry season, when the temperature is from 14° to 16° C.

The figures quoted above, therefore, show that *Mitrastemon* grows only in temperatures ranging between 10° and 20° C and principally during the dry season when the activities of the host tree are at a minimum. When the plants are in bloom honey-like fluids with a special odor are secreted at the basal portion of the peduncles. These fluids attract various insects and a large number of little birds. In Kagosima the local name of the parasite is "Hanasii-no-Titi," which signifies "milk of meziro" (the "meziro" is a favorite little bird, *Zosterops japonica* T. & Z.). From Taiwan it is reported that one of the insects attracted is *Vespa magnifica* var. *nobilis* Sonan, and in Sumatra a species of *Drosophila*. In Mexico the writer has secured two species of insects from the plant, but they have not yet been identified. Pollination is, naturally, accomplished by these birds and insects.

KEY TO THE SPECIES

I have made mention of the three species of *Mitrastemon* which we know from Japan and Sumatra, namely, *M. Yamamotoi* Mak., *M. Kawa-Sasakii* Hay., and *M. Kanehirai* Yam. Now, however, according to Makino, the last two species are recognized as merely varieties of the first (10).

A key for the identification of these species and varieties follows.

Plant height 3–6 cm.; scales in 6 pairs, opposite, imbricate. (In Japan proper
  and Okinawa.)   *M. Yamamotoi* **Mak.**
Plant height 15 cm.; scales oblong, in 6 pairs. (In Taiwan and Sumatra.)
                                           M. Yamamotoi var. *Kawa-Sasakii* (Hay.) **Mak.**
Plant height 6–8 cm.; scales in 12 pairs; 4-angled, obconic in form. (In
  Taiwan.)   *M. Yamamotoi* var. *Kanehirai* (Yam.) **Mak.**
Plant height 4–12 cm.; scales broad, triangular, in 7 pairs; stigma conic;
  fruits appressed, cylindric. (In southern Mexico.)   *M. Matudai* **Yam.**

SYSTEMATIC POSITION

The genus *Mitrastemon* is a very peculiar group, widely different from all other known flowering parasites. It is possible to find some affinities for it in each of the following five families: *Nepenthaceae* of Malaya, *Rafflesiaceae* of Indonesia, Africa, and tropical America, *Hydnoraceae* of Africa and tropical America, *Balanophoraceae*, and *Aristolochiaceae*. Affinity with the *Nepenthaceae* appears in (1) the superior ovary, and (2) the single perianth, but the structure of the flower and the fruit differs widely.
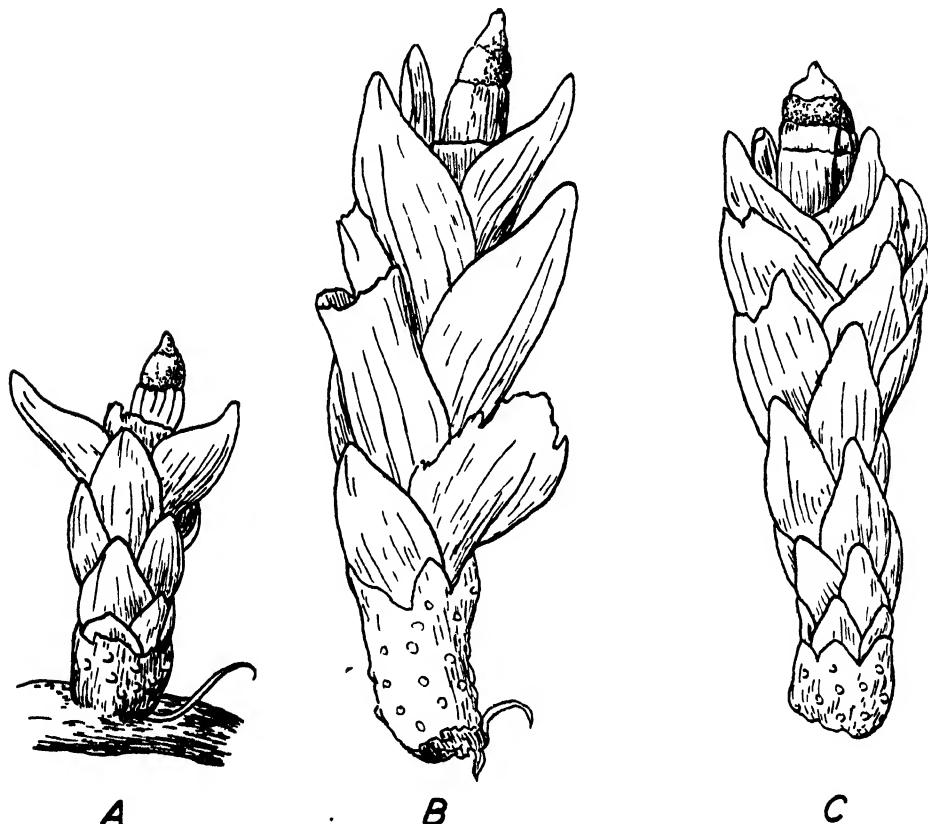
FIG. 10. A, *Mitrastemon Yamamotoi* Mak.; B, *Mitrastemon Yamamotoi* var. *Kawa-Sasakii* (Hay.) Mak.; C, *Mitrastemon Yamamotoi* var. *Kanehirai* (Hay.) Mak. All natural size.

There are many similar morphological and anatomic characters which point to the *Rafflesiaceae*, as has been pointed out and discussed by Hayata— for instance, (1) it is a parasite, (2) is hermaphrodite, (3) is leafless but bears scales, and (4) has a one-celled ovary with parietal placentae. Differences are seen in (1) the superior perianth, (2) anthers growing on single

or several lines, (3) the inferior ovary, and (4) the disk-like torus on which the anthers grow, etc.

As to the *Hydnoraceae* the points of likeness are (1) the parasitic nature of the plants, (2) the single hermaphrodite flowers, and (3) the perianth-tube. But they differ in (1) the 3–4-lobed perianth, (2) the anthers placed in 1 or 2 series attached to the perianth-tube, and (3) the epigynous, sessile filaments, etc.

With the *Balanophoraceae* there is close affinity in (1) the parasitic nature, (2) the single, unbranched stems with scales, (3) the basal volva, and (4) the hypogynous androecium. But there is dissimilarity in (1) the multiflowered or saraceous peduncle, (2) the mostly diclinous flowers, (3) the 3–4-lobed perianth, (4) the 1–8-celled anther, and many other differences in the principal structure of the flower.

Finally, it resembles the *Aristolochiaceae* in its (1) coherent perianth, (2) baccate fruits, (3) anatropous ovules; but the latter family is easily distinguished by its (1) superior perianth, (2) epigynous flower, and (3) leafy and not parasitic nature.

Above all, our genus is characterized by its (1) mitriform, united, connate stamens, (2) hypogynous flowers, and (3) anatropous ovules, in which it is distinct from all the other families. After considering the extent of the differences from the above five families, we are forced to the conclusion that *Mitrastemon* forms an independent family, as suggested by Makino and recently approved by Honda and Sakisaka in their *Plant Taxonomy of Nippon* (12, 14). The *Mitrastemonaceae* should be placed between the *Rafflesiaceae* and the *Hydnoraceae*.

DISTRIBUTION

Since the first discovery of *Mitrastemon* unusual attention has been given to this group of parasites, and specimens have been collected in many localities where it was hitherto not known to exist. The genus is now reported from the Tokusima prefecture, northeast of Koti, where it was first discovered, on the same island of Sikoku, and this is its northern limit. In the Koti prefecture it has been collected extensively in many *Shiia* zones; also, it is newly reported from the Miyazaki prefecture on the island of Kyusyu, northeast of the Kagosima locality previously mentioned. In the Kagosima prefecture it is now also widely known in the *Shiia* zone, and it is also reported on the small islands of Amami-osima and Yakusima of the same Kagosima prefecture. The range of distribution extends south to Okinawa, a prefecture of the Ryukyu Archipelago. In all these localities only the one species, *M. Yamamotoi,* is known.

In Taiwan it is widely known from the north to the south in the temperate zone of *Fagaceae,* but only in the varieties *Kawa-Sasakii* and *Kanehirai.* The

first of these varieties ranges as far south as Sumatra. At Rengeti, in the center of Taiwan, both of the varieties have been found practically inter-mingled, but always on hosts of separate species.
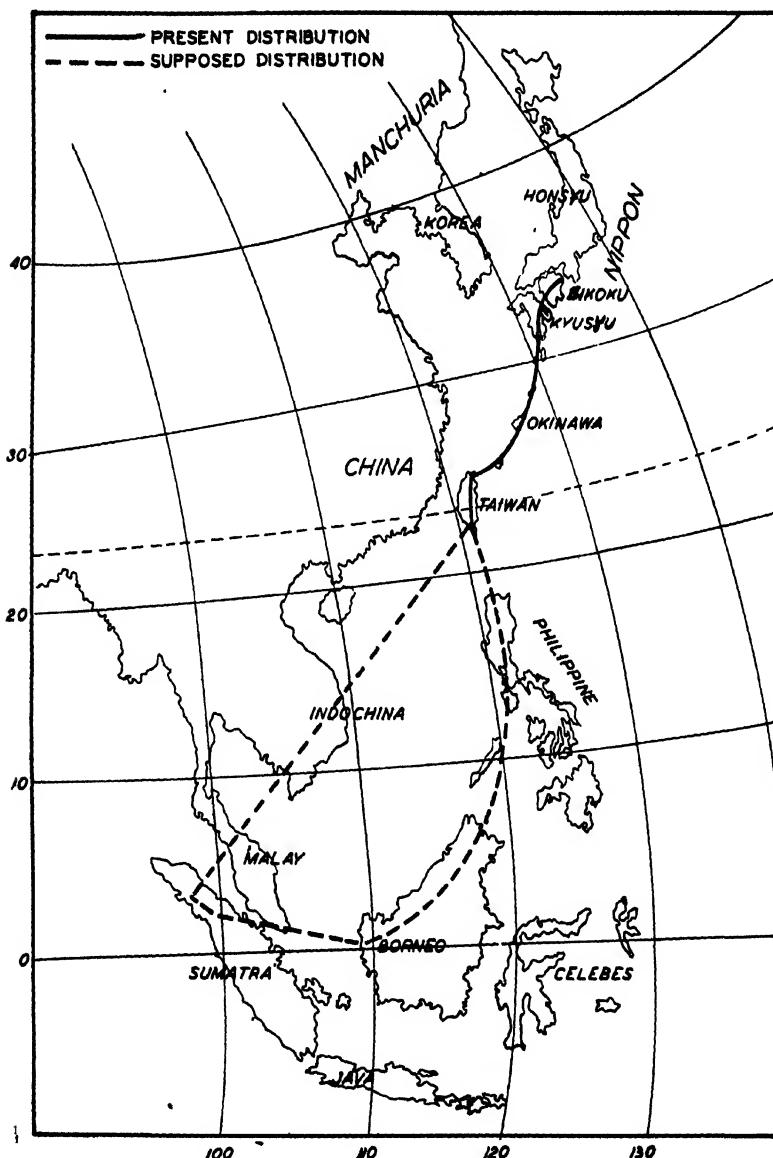
FIG. 11. Distribution of *Mitrastemon* in eastern Asia.

In the near future it may possibly be found in Malaya, Borneo, and the Philippines, in which case the geographic distribution of *Mitrastemon* would stretch from Sumatra to southern Japan, forming an arc in eastern Asia (fig. 11).

Very recently it has been reported that *Mitrastemon Matudai* has been found by Paul C. Standley at Altaverapaz, Guatemala (11–14).

The new appearance of *Mitrastemon* in southern Mexico and in Guatemala, beyond the vast Pacific Ocean, presents a great puzzle to geobotanists. It will prove exceedingly difficult to harmonize this with the generally accepted tenets of the Darwinian theory of evolution.

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NOTES ON THE COMPOSITAE OF THE NORTHEASTERN UNITED STATES—V. ASTEREAE

ARTHUR CRONQUIST

This series of papers is being presented in order to validate certain nomenclatural changes necessitated in preparing the treatment of the *Compositae* for Dr. Gleason's new illustrated flora of the northeastern states, and to allow for more ample discussion of some of those changes than is possible within the confines of a flora. Several previous numbers in the series have appeared in Rhodora. The present paper deals principally with *Aster*, but also includes the other *Astereae* except *Solidago*, on which a separate paper has been prepared for Rhodora.

Several years ago, when I was revising the western representatives of the *Aster foliaceus* complex, I noticed that the eastern American plants which had been referred to *A. foliaceus* Lindl. could generally be recognized at a glance, purely by their habit. These specimens, furthermore, were in some respects (such as the commonly toothed leaves) nearer to *A. douglasii* Lindl. than to *A. foliaceus*.

Upon re-examination of these eastern plants, I am convinced that they should be distinguished specifically from their western relatives. The habit difference previously noted is largely·a matter of the conspicuously crowded cauline leaves of the more common eastern phase of the species. Another significant character, although it is not always well shown in herbarium specimens, is that *A. foliaceus* tends to have well developed basal leaves (often on separate short shoots) which are larger and more petiolate than those above, while eastern plants tend to have the lower leaves similar to or smaller than those above, without any basal cluster. The lower leaves may sometimes be deciduous 'before flowering in *A. foliaceus,* but are uniformly so in eastern plants.

Another factor to be considered in reaching a decision on the status of the eastern plants is that. their greatest similarity is not to *A. foliaceus* proper, but to apparent hybrids between that species and *A. douglasii.* Inclusion of the eastern plants in either of these species would seemingly necessitate the reduction of the other, although each of them is already broadly defined and highly variable.

Three specific names should be considered before a new name is proposed for the eastern plants under discussion. *Aster johannensis* Fern., from Lake St. John, resembles *A. novi-belgii* L. in habit, but has narrower, more appressed phyllaries, much as in *A. junciformis* Rydb. An isotype at New York

seems reasonably typical of *A. novi-belgii*. Two collections from James Bay (*Dutilly 13347* and *13479*), where *A. novi-belgii* is otherwise unknown, closely resemble the type of *A. johannensis*, suggesting that it may prove to be more than merely an extreme form of *A. novi-belgii*. In any case, these plants are not the same as the eastern species that has been referred to *A. foliaceus*.

Aster anticostensis Fern. appears to be either an unusual form of *A. novi-belgii* or possibly a hybrid involving that species.

Aster gaspensis Vict. is apparently a good species, with relatively narrow, mostly entire, scarcely or not at all auriculate leaves, and with more elongate inflorescence and less leafy involucre than the eastern plants of *"A. foliaceus."* A number of collections from the vicinity of Lake Mistassini which have been referred to *A. foliaceus* seem more properly to belong to *A. gaspensis*.

Since there seems to be no available specific name for the eastern relatives of *A. foliaceus* under discussion, I am elevating one of the several proposed varieties to specific rank.

Aster crenifolius (Fern.) Cronquist, comb. nov. *A. foliaceus* var. *crenifolius* Fern. Rhodora **17**: 15. 1915.

Most of the specimens of *A. crenifolius* have conspicuously crowded leaves, with the middle and upper internodes mostly 5–30 (or 35) mm. long, but a number of plants, chiefly from more protected habitats such as alluvial stream-bottoms, have more distant leaves, with the middle and upper internodes mostly 3–6 cm. long. These latter specimens also differ in several scarcely tangible ways, giving the impression of being much laxer than the more typical plants. Although the ranges of the two are nearly or quite coextensive, it seems desirable to distinguish them varietally.

Aster crenifolius (Fern.) Cronquist var. **crenifolius** (Fern.) Cronquist, comb. nov. *A. foliaceus* var. *crenifolius* Fern. Rhodora **17**: 15. 1915. *A. foliaceus* var. *subpetiolatus* Fern. loc. cit., a robust form.

Aster crenifolius (Fern.) Cronquist var. **arcuans** (Fern.) Cronquist, comb. nov. *A. foliaceus* var. *arcuans* Fern. Rhodora **17**: 14. 1915. The laxer, less leafy plants referred to above.

Aster foliaceus var. *sublinearis* Griscom & Eaton is in my opinion merely an extreme form of *A. novi-belgii*, with more than usually leafy involucre. *A. foliaceus* var. *subgeminatus* Fern., still known only from the type collection in Newfoundland, is a puzzling plant which I do not feel able to place at present. Were *Aster ciliolatus* Lindl. known to occur in Newfoundland, I would be inclined to consider *A. foliaceus* var. *subgeminatus* a hybrid between that and *A. crenifolius*. Its status, in any case, has no apparent bearing on the distinctness of *A. crenifolius* from *A. foliaceus*.

It may also be pointed out that most of the material from Nova Scotia

referred to *A. foliaceus* by Shinners (Rhodora **45**: 344–351. 1943) is in my opinion *A. novi-belgii*, as is also *A. rolandii* Shinners.

It has been customary, in recent years, to separate *Aster falcatus* Lindl. from *A. commutatus* (T. & G.) A. Gray by its thinner, less squarrose, more acute involucral bracts, sparser pubescence, and more northern distribution. The morphologic characters are intangible and inconstant, and are scarcely to be made out at all without comparison of an extensive suite of specimens. The supposed difference in range is largely illusory. Specimens differing in the technical features noted look very much alike in other respects. Furthermore, there is no suggestion of discontinuity, or even modal distribution, in these supposedly distinctive features. I find it necessary, then, to combine the two species under the earlier name, *A. falcatus* Lindl.

In *Aster falcatus*, as in the closely related *A. ericoides* L., the majority of the specimens may be placed in two categories according to the orientation of the cauline pubescence, with only a relatively few intermediates. In spite of the fact that their ranges are essentially co-extensive, it therefore seems desirable to give these pubescence-types varietal recognition.

ASTER FALCATUS Lindl. var. **falcatus** Cronquist, var. nov. *A. falcatus* Lindl. in DC. Prodr. **5**: 241. 1836, sensu stricto. Hairs of the stem mostly appressed or closely ascending.

ASTER FALCATUS Lindl. var. **crassulus** (Rydb.) Cronquist, comb. nov. *A. crassulus* Rydb. Bull. Torrey Club **28**: 504. 1901. Hairs of the stem spreading.

As has been pointed out by Mrs. Nelson (Rhodora **35**: 323–325. 1933), cordilleran plants of "*A. ericoides*" have the stems clustered from a short rhizome or branched caudex. Mrs. Nelson was apparently unaware, however, that typical *A. ericoides* L., as it occurs in the eastern United States, regularly has the stems arising from well developed creeping rhizomes. It seems not unlikely that the cordilleran plants which have been referred to *A. ericoides* should properly be treated as a distinct species, to be distinguished by their cespitose habit and some less tangible or less constant features recorded by Mrs. Nelson, but I hesitate to do so at this time, for occasional cordilleran plants of otherwise apparently not unusual *A. falcatus* are also cespitose instead of creeping-rhizomatous. Until the significance of the cespitose versus creeping-rhizomatous habit in this group can be studied in greater detail, and especially in view of the fact that it is doubtful that there is an available specific name properly applicable to the cordilleran phase of "*A. ericoides*," I think it is better not to propose any formal taxonomic segregation.

In comparing specimens of *Aster paludosus* Ait., preparatory to drawing up a description, I noticed an extremely wide variation in the shape of the inflorescence. Some specimens showed a narrow, elongate, racemiform or

spiciform inflorescence, while others had the heads more loosely arranged in a broader, corymbiform inflorescence. These latter specimens, furthermore, had the inflorescence more obviously hairy than the others. Sorting the specimens on these characters revealed that those with the corymbiform inflorescence occur from North Carolina to Florida and Alabama, while the others range from Missouri and Kansas to Texas and Alabama. In Alabama, where the ranges meet (as, for example, in Cullman County), there are doubtful specimens; otherwise the distinction is seldom difficult. A good case might be made out for distinguishing the two phases as separate species, but they are obviously very closely related, and I think the taxonomic needs are in this case best met by use of the subspecific category.

In his treatment of the group for Small's flora of the southeastern United States, Alexander restricted the name *A. paludosus* to the eastern, more hairy plant, and divided the remainder of the group into four new species. These new species were distinguished from each other chiefly by the presence or absence of creeping rhizomes and the degree of imbrication of the involucral bracts. Both of these characters are frequently valuable in the genus, but in this case they seem to be independently variable and without taxonomic significance. Since the cultivated plants on which the original *Aster paludosus* of Aiton was based were stated to have come from Carolina, Alexander's selection of the typical element of the species may well be adhered to.

ASTER PALUDOSUS Ait. subsp. **paludosus** Cronquist, subsp. nov. *A. paludosus* Ait. Hort. Kew. **3**: 201. 1789, sensu stricto.

The more western, less hairy plants, with narrower and more elongate inflorescence may be known as *Aster paludosus* Ait. subsp. **hemisphaericus** (Alexander) Cronquist, comb. nov. (*A. hemisphaericus* Alexander in Small, Fl. S.E. U.S. 1391. 1933. *A. pedionomus* and *A. gattingeri* Alexander, loc. cit. *A. verutifolius* Alexander, ibid., p. 1392).

Dr. L. H. Shinners, among others, has pointed out that the name *Aster paniculatus* Lam. (1783) is antedated by *Aster paniculatus* Mill. (1768) and thus is illegitimate. The next available name which applies with reasonable certainty to Lamarck's species, so far as I am aware, is *A. simplex* Willd. Enum. Hort. Berol. 887. 1809. This is the relatively broad-leaved phase which Dr. Wiegand treated as *A. paniculatus* var. *simplex* (Willd.) Burgess. It becomes necessary, then, to find a varietal name for the narrow-leaved, chiefly northeastern phase of the species, which Wiegand treated as *A. paniculatus* proper. This is provided in *A. simplex* var. **ramosissimus** (T. & G.) Cronquist, comb. nov., based on *A. tenuifolius* β *ramosissimus* T. & G. Fl. N. Am. **2**: 132. 1841.

The small-headed midwestern plants which Dr. Wiegand segregated from *A. paniculatus* as *A. interior* Wieg. do form a definable entity, but pass readily into the larger-headed forms, and differ in no other apparent charac-

ter. I think it proper, then, to reduce them to varietal status. Since, as Dr. Wiegand showed, the type of *A. carneus* γ *ambiguus* T. & G. may be of hybrid origin rather than representing pure *A. interior*, it seems best to retain Wiegand's epithet in making the new combination.

Aster simplex Willd. var. **interior** (Wieg.) Cronquist, comb. nov. *A. interior* Wieg. Rhodora **35**: 35. 1933.

The nomenclaturally typical variety of *A. simplex* may be known as *Aster simplex* Willd. var. **simplex** Cronquist, var. nov., based on *A. simplex* Willd. Enum. Hort. Berol. 887 (1809), sensu stricto.

Aster laevis L. var. **laevis** Cronquist, var. nov. *A. laevis* L. Sp. Pl. 876. 1753, sensu stricto.

Aster patens Ait. var. **patens** Cronquist, var. nov. *A. patens* Ait. Hort. Kew. **3**: 201. 1789, sensu stricto.

Aster pilosus Willd. var. **pilosus** Cronquist, var. nov. *A. pilosus* Willd. Sp. Pl. **3**: 2025. 1803, sensu stricto.

Aster puniceus L. var. **puniceus** Cronquist, var. nov. *A. puniceus* L. Sp. Pl. 875. 1753, sensu stricto.

Professor Fernald has recently pointed out (Rhodora **42**: 492–495. 1940) that, although northern plants referred to *A. humilis* Willd. or *A. umbellatus* var. *latifolius* Gray are merely extreme specimens of *A. umbellatus*, the southern specimens so referred belong to a well-characterized entity with larger and firmer involucres and usually broader leaves. Another character of the southern plants, not mentioned by Fernald, is so distinctive that I feel they should be specifically separated. Their heads are only 6–19-flowered, with 2–7 rays and 4–12 disk flowers. Furthermore, the general aspect of the southern plants is as nearly that of *A. infirmus* Michx as of *A. umbellatus*, and in the character of the involucre they much more closely resemble the former than the latter. In *A. infirmus* the heads are 24–45-flowered, with 6–9 rays and 18–36 disk flowers. There is thus a clear break in number of disk flowers between the southern plants referred to *A. umbellatus* var. *latifolius*, on the one hand, and *A. umbellatus* and *A. infirmus* on the other.

A further, perhaps minor consideration is that the inclusion of these southern plants in *A. umbellatus* would remove one of the most satisfactory key-characters to distinguish that species from the universally recognized *A. infirmus*, for, while the achenes of *A. umbellatus* are hairy, and those of *A. infirmus* glabrous, those of the third group may be either glabrous or slightly hairy. The name *A. humilis* Willd. was based on northern plants which, as Professor Fernald has shown, are not separable from *A. umbellatus*. The only available specific name for the southern group, so far as I am aware, is *Aster sericocarpoides* (Small) K. Schum., based on *Doellingeria sericocarpoides* Small.

A further investigation of *Aster umbellatus* reveals that the hairy west-

ern plants which have generally been treated as *A. umbellatus* var. *pubens* Gray have a reduced number of flowers, much as in *A. sericocarpoides*. The heads are mostly 12–22-flowered, with 4–7 rays and 8–15 disk flowers, instead of 23–54-flowered, with 7–14 rays and 16–40 disk flowers. Although there is some slight failure in the distinction here, it is no greater than in many other cases of generally recognized species in *Aster*, and consistency would suggest that specific recognition is desirable. The name *Aster pubens* is pre-occupied by a proposal of Otto Kuntze. Since Kuntze's name was based on a species of *Solidago*, I feel that the very similar name here proposed will cause no serious confusion.

Aster pubentior Cronquist, nom. nov. *A. umbellatus* var. *pubens* Gray, Syn. Fl. 1²: 197. 1884.

Certain northeastern specimens of *Aster umbellatus*, chiefly from Newfoundland and Nova Scotia, have the stem, involucres, and lower surfaces of the leaves puberulent, much as in *A. pubentior* but more coarsely so. They also tend to be smaller than typical *A. umbellatus*, with fewer heads. In other respects, however, they appear to be quite typical of the species, and no clear line can be drawn to separate them. It may eventually be desired to treat them as a separate variety, but there are so many intermediates that I am not prepared to do so at this time.

The four species of *Aster* § *Doellingeria* here discussed may usually be identified by the following key.

1. Heads mostly 6–22-flowered, with 2–7 rays and 4–15 disk flowers.
    2. Involucre 3.5–5 mm. high, its bracts puberulent, generally long triangular and acute; leaves more or less puberulent over the surface beneath; n. Mich. to Neb. and Alta.   *A. pubentior.*
    2. Involucre 4–7 mm. high, its bracts glabrous or nearly so, at least the inner ones oblong or linear oblong and obtuse or rounded; leaves usually glabrous or merely puberulent along the veins, beneath, occasionally appressed puberulent over the surface; N. C. to Fla. and La.   •   *A. sericocarpoides.*
1. Heads mostly 23–54 flowered, with 6–14 rays and 16–40 disk flowers.
    2. Achenes more or less strigose or puberulent; plant normally with creeping rhizomes; involucre 3–5 mm. high, its bracts relatively thin and slender; heads mostly 30–300 or more in a usually relatively dense and more or less flat-topped inflorescence, sometimes fewer in northern or depauperate forms; Newf. to n. Ga., west to Minn. and n. Ill.   *A. umbellatus.*
    2. Achenes glabrous; plants without creeping rhizomes; involucre 4.5–7 mm. high, its bracts relatively broad and firm; heads mostly 5–30 in an open inflorescence, rarely as many as 75; Mass. to Ga. and Ala.   *A. infirmus.*

Until the publication of the first part of the second volume of the *Genera Plantarum* in 1873, it was customary at least in America to regard the various *Aster*-segregates as valid genera. Asa Gray rather reluctantly followed Bentham's sweeping reduction of most of the segregates, and further pointed out that if these were to be reduced, *Brachyactis* would have to follow.

While the extended definition of *Aster* has certainly not been uniformly accepted, it has been followed by the majority of American taxonomists since Gray's adoption of it, more especially so since the swing away from the splitting vogue of the first quarter of this century. The Benthamian definition, with Gray's addition of *Brachyactis*, was also adopted by Hoffman in his treatment of the Compositae for the *Natürlichen Pflanzenfamilien*.

Although I do not wish to become involved at this time in a discussion of the propriety of the extended definition of *Aster*, I must support it, with some misgivings, at least until a thorough study of the old-world as well as the American species provides a reasonable basis on which all species can be referred either to *Aster* proper or to a morphologically definable segregate. (It may be mentioned that *Leucelene* might perhaps be allowed to stand without disturbing the others.)

If *Aster* is to be accepted in the extended sense, however, *Sericocarpus* must follow the other segregates to intra-generic rank. The only basis on which the several species of *Sericocarpus* might be distinguished generically is their narrow, relatively few-flowered heads. *Sericocarpus linifolius* (L.) B.S.P., the type of the genus, has 3–6 rays and 5–10 disk flowers. Another northeastern species, *S. asteroides* (L.) B.S.P., has 4–8 rays and 9–20 disk flowers. Two species of *Aster* § *Doellingeria* reach this low level, however, as noted previously: *Aster sericocarpoides* has 2-7 rays and 4–12 disk flowers and *A. pubentior* has 4–7 rays and 8–15 disk flowers. *Aster perelegans* Nels. & Macbr. (= *Eucephalus elegans* Nutt.) sometimes has as few as 4 rays and 8 disk flowers to a head. At least two species of *Aster* proper also have heads with scarcely more flowers than *Sericocarpus*. *A. parviceps* (Burgess) Mack. & Bush has 18–32-flowered heads, with only 6–14 disk flowers, and *A. depauperatus* (Porter) Fern. has 15–32-flowered heads, with 8–17 disk flowers. *Aster divaricatus* L., of the section *Biotia*, although it has more numerous disk flowers, frequently has only 5 rays.

Thus we see that a reduction in number of flowers per head has occurred in several groups in *Aster*, and that this reduction has proceeded no farther in *Sericocarpus* than in some of the other groups. All the other characters of *Sericocarpus*, including the densely hairy achenes (compare *A. novae-angliae* and *A. concolor*) and the broad, chartaceous, shortly green-tipped involucral bracts (compare *A. surculosus*, *A. gracilis*, and § *Biotia*), are within the range of variation of *Aster*. There remains no basis on which *Sericocarpus* can be segregated.

Of the two species of *Sericocarpus* in the manual range, *S. linifolius* takes the name *Aster solidagineus* Michx., since the name *Aster linifolius* is preoccupied. *Sericocarpus asteroides* apparently has no valid name in *Aster*, since the name *Aster asteroides* is preoccupied, and both *A. marilandicus* Michx. and *A. conyzoides* Willd. were illegitimate substitutes for the Lin-

naean *Conyza asteroides*, proposed at a time when the epithet *asteroides* was still available in *Aster*. Associating the species with Linnaeus, I therefore propose the name **Aster paternus** Cronquist, based on *Conyza asteroides* L. Sp. Pl. 861 (1753).

An interesting nomenclatural question might be raised as to whether the exception given in article 69 of the International Rules of Botanical Nomenclature to the otherwise blanket rejection of illegitimate names could be so construed as to make it possible to take up Michaux's name *Aster marilandicus* for the plant under discussion. The pertinent part of article 69 reads, "Where a new epithet is required, an author may, if he wishes, adopt an epithet previously given to the group in an illegitimate combination, if there is no obstacle to its employment in the new position or sense." The example given thereafter pertains to a transfer to one genus of an epithet illegitimate in another. Two competent students of the Rules have assured me that the legitimacy of *Aster marilandicus* would now be restored if I were to publish it anew, using the same type as Michaux (that is, basing it on *Conyza asteroides* L). Another equally competent nomenclaturist has taken the stand that the provision in article 69 is meant to apply only to cases where a transfer is involved, as illustrated in the example given for it in the Rules. The point might well be considered by the next International Congress.

Professor Fernald has shown that the many-headed plant of the interior that has passed as *Boltonia asteroides* (L.) L'Hér. is not the same as true *B. asteroides* of the coastal states, and is not specifically separable from *B. latisquama* Gray. The difference between the coastal few-headed plant and the inland many-headed plant does not seem to me to be nearly so constant as Fernald makes out, however, so that all should be combined under the heading of *B. asteroides*.

BOLTONIA ASTEROIDES (L.) L'Hér. var. **asteroides** Cronquist, var. nov. *B. asteroides* (L.) L'Hér. Sert. Angl. 16. 1788, sensu stricto. *Matricaria asteroides* L. Mant. 116. 1767.

BOLTONIA ASTEROIDES (L.) L'Hér. var. **recognita** (Fern. & Griscom) Cronquist, comb. nov. *B. latisquama* Gray var *recognita* Fern. & Griscom, Rhodora **42**: 491. 1940.

BOLTONIA ASTEROIDES (L.) L'Hér. var. **latisquama** (Gray) Cronquist, comb. nov. *B. latisquama* Gray, Am. Jour. Sci. II. **33**: 238. 1862.

Chrysopsis villosa is a highly variable species from which many segregates have been proposed. Some of these appear very distinct in the extreme form, but apparently all are connected by numerous intergrades.

CHRYSOPSIS VILLOSA (Pursh) Nutt. var. **villosa** Cronquist, var. nov. *Chrysopsis villosa* (Pursh) Nutt. Gen. **2**: 151. 1818, sensu stricto. *Amellus villosus* Pursh, Fl. Am. Sept. 564. 1814.

CHRYSOPSIS VILLOSA (Pursh) Nutt. var. **angustifolia** (Rydb.) Cronquist, comb. nov. *C. angustifolia* Rydb. Bull. Torrey Club **37**: 128. 1910.

CHRYSOPSIS VILLOSA (Pursh) Nutt. var. **camporum** (Greene) Cronquist, comb. nov. *C. camporum* Greene, Pittonia **3**: 88. 1896.

CHRYSOPSIS GRAMINIFOLIA (Michx.) Ell. var. **graminifolia** Cronquist, var. nov. *Chrysopsis graminifolia* (Michx.) Ell. Bot. S. C. & Ga. **2**: 334. 1824, sensu stricto. *Inula graminifolia* Michx. Flor. Bor. Am. **2**: 122. 1803.

When I transferred *Erigeron canadensis* L. and its allies to *Conyza*, several years ago, I accepted Robinson's treatment (Rhodora **15**: 205–209. 1913) in which he recognized the coastal *E. pusillus* Nutt. as specifically distinct from *E. canadensis*. In working over the group for the illustrated flora, I find a considerable number of intermediates. Since the plants are habitally very similar, and further since there is a chiefly western phase of *E. canadensis* which has the glabrous stem of *E. pusillus*, without sharing its other features such as the minutely purple-tipped involucral bracts, I think it is better to combine them all in one species.

All of the species which Linnaeus referred to *Conyza* in the *Species Plantarum* have now been transferred to other genera, so that the use of the name in the traditional sense can be continued only through permissive action by an international botanical congress. As noted by Bentham in the *Genera Plantarum*, the familiar application of the name was established by Lessing in his *Synopsis generum Compositarum* in 1832. The name *Eschenbachia* Moench. is available for the group, but it is relatively little-known, and only a few species have been formally transferred to it. Until the matter has been considered by a botanical congress, I intend to continue to use the name *Conyza*.

CONYZA CANADENSIS (L.) Cronquist var. **canadensis** Cronquist, var. nov. *Erigeron canadensis* L. Sp. Pl. 863. 1753.

CONYZA CANADENSIS (L.) Cronquist var. **pusilla** (Nutt.) Cronquist, comb. nov. *Erigeron pusillus* Nutt. Gen. **2**: 148. 1818. *Conyza parva* Cronquist, Bull. Torrey Club **70**: 632. 1943.

CONYZA CANADENSIS (L.) Cronquist var. **glabrata** (A. Gray) Cronquist, comb. nov. *Erigeron canadensis* var. *glabratus* A. Gray, Pl. Lindh. **2**: 220. 1850.

GRINDELIA SQUARROSA (Pursh) Dunal var. **squarrosa** Cronquist, var. nov. *Grindelia squarrosa* (Pursh) Dunal in DC. Prodr. **5**: 315. 1836, sensu stricto. *Donia squarrosa* Pursh, Fl. Am. Sept. 559. 1814.

THE NEW YORK BOTANICAL GARDEN
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STATUS AND DISTRIBUTION OF CAREX NEUROCHLAENA HOLM

MAXIMILIAN G. DUMAN

Kükenthal (1909) places *Carex neurochlaena* Holm (1904) in his "species dubiae." Mackenzie (1931) regards it, together with *C. glareosa* var. *amphigena* Fernald, as a synonym of *C. marina* Dewey. But both Kükenthal and Fernald refer *C. marina* Dewey to *C. Heleonastes* Ehrh. The type specimen (*Macoun 53879*) of *C. neurochlaena* Holm, collected above Rink Rapids, Yukon River, Yukon, is preserved in the Langlois Herbarium at Catholic University, Washington, D. C. This specimen is accompanied by Holm's original drawings of the figures published with its description. Another specimen of *Macoun 53879* (isotype) in the Herbarium of the National Museum of Canada, Ottawa, is annotated by Mackenzie as "*C. Heleonastes*" (fide Porsild 1943).

The validity of *C. neurochlaena* appeared to rest on firmer ground when this species was collected at six localities in Eastern Arctic Canada by Dutilly, O'Neill and the author, and was so reported by Duman (1941). Later A. E. Porsild (1943) reported it from seven localities in the Northwest Territories, stating that its reduction to synonymy "seemed entirely unjustified," and that it "was previously known only from the type locality."

A search through the collections of closely related species in the Langlois Herbarium, the Philadelphia Academy of Science, and the Carnegie Museum, Pittsburgh, failed to reveal any additional specimens. It is significant also that *C. Heleonastes* Ehrh., which it resembles more than any other, and next to which it was originally placed by Holm, has not been reported from the arctic, while all the collections of *C. neurochlaena* are from localities with essentially arctic climates.

The following keys will help distinguish *C. neurochlaena* from related northern species.

Lateral spikes gynaecandrous, lowest spike occasionally pistillate; perigynia elliptic, 1 mm. wide, 2.5–3 mm. long, green becoming brown, subcoriaceous, attenuate at both ends; anthers 0.9–1 mm. long. *C. neurochlaena* Holm.

Lateral spikes pistillate; perigynia obovoid, 1.3–1.9 mm. wide, 2–3 mm. long, pale brown or drab, membranaceous, abruptly beaked; anthers 2 mm. long. *C. glareosa* Wahl. (var. *amphigena* Fernald).

Spikes 4–7 mm. long, few flowered, roundish; perigynia broadly elliptic, green becoming brown, subcoriaceous, attenuate at both ends; anthers 0.9–1 mm. long. *C. neurochlaena* Holm.

Spikes 5–10 mm. long, few- to many-flowered, oblong to suborbicular; perigynia obovate, brownish-yellow, membranaceous, round-tapering at the base, rather abruptly

contracted at the apex into a short prominent beak; anthers 1.5–2 mm. long. *C. bipartita* Bellardi ex All.

Perigynia elliptic, 1 mm. wide, 2.5–3 mm. long, gradually smooth-beaked; anthers 0.9–1 mm. long; margins of the glumes clasping the perigynia. *C. neurochlaena* Holm.

Perigynia ovate, 1.5 mm. wide, 3 mm. long, abruptly scabrous-beaked; anthers 1.5–2 mm. long; margins of the glumes not clasping the perigynia. *C. Heleonastes* Ehrh.

Distribution of *Carex neurochlaena* Holm. YUKON: Yukon River above Rink Rapids, *Macoun 53879*. MACKENZIE: Mackenzie River Delta, *Porsild 2331, 2439;* Arctic Coast, *Porsild 2564, 2851;* Eskimo Lake Basin, *Porsild 2970;* Great Bear Lake, *Porsild 5031, 5115*. NORTH HUDSON BAY: Repulse Bay, *Duman 2032, 2049a1*. WEST HUDSON BAY: Baker Lake, *Dutilly 6227a;* Chesterfield, *Duman 1846, 1848, 1882b, Dutilly 6755;* Churchill, *Duman 1262, 1292, 1341, 1346, 1359, 1367;* Churchill (West of River), *Duman 1467, 1498, Dutilly 6537, 6574*. HUDSON STRAIT: Wakeham Bay, *Dutilly, O'Neill & Duman 87253*.

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EUPHORBIA MACULATA L.

Leon Croizat

An entirely novel application of the Linnean *Euphorbia maculata* and
E. Chamaesyce was sponsored by Wheeler (Contr. Gray Herb. **127**: 74. 1939;
Rhodora **43**: 265. 1941), which aroused misgivings in the mind of prudent
taxonomists. These two names had endured well over a century in a sense
agreeing with current usage, and it was not certain that absolute necessity
required a change.

In a previous study (Bull. Torrey Club **72**: 312. 1945), I endeavored to
show that the transfer of *E. Chamaesyce* L. to a species universally known
as *E. prostrata* Ait. was uncalled for, and that even a Chicagoan fragment
of the "Linnean type" was not enough of a lever to effect revolutionary
alterations. After the publication of this work, my advice was privately
sought on whether I had objections against using *E. maculata* in the
Wheelerian sense. I answered that I could not indorse this sense.

I would have stopped here, if Fosberg had refrained (Rhodora **48**: 197.
1946) from supporting Wheeler. I had felt that controversy was unneces-
sary because Svenson's note on *E. maculata* (Rhodora **47**: 273. 1945) gave
fair warning to hopeful users of this binomial in the Wheelerian manner that
they had better reinvestigate the question. It meant nothing to me that Sven-
son's grounds and mine were entirely different so long as the result could
be the same for taxonomy.

It is clear that Fosberg is no better aware than Wheeler of the real status
of the issue, which requires final restatement. The point at issue is fact, and
the reader may embroider further at his own pleasure.

Boissier's monograph of the *Euphorbieae* (in DC. Prodr. **15**(2): 3. 1862)
is still viable, and few taxonomists will be inclined to slight the diligence and
trustworthiness of this author Boissier's treatment of *E. maculata* (op. cit.
46) reads, "156. *E. maculata* (L. sp. pl. I. n. 21 et hb.! non L. mant.). . . .
In Americâ boreali a Canadâ ad Floridam (Chapman!) et Texas! . . . .
E. supina Rafin. dec. pl. . . . ."

It is manifest that: (1) Boissier diligently consulted the Linnean her-
barium before 1862; (2) elected one particular specimen out of this her-
barium as the type, which agreed with the traditional application of the
binomial; (3) further investigated, reaching the conclusions that the Lin-
nean concept of the *Mantissa* was no longer the same as that of the *Species
Plantarum*.

Vague beliefs exist that the botanists of the Old World never had the
perfect understanding of "type" vouchsafed to those of the New, which it

would be instructive to discuss. Whatever the details, Boissier effected in this treatment an iron-clad "typification" of the most modern pattern both under the International and the American Code of Nomenclature.

Informed of this treatment, a critical taxonomist mistrusts forthwith a photograph of the Linnean "type" of *E. maculata* disagreeing with the Boisserian understanding of it. The Linnean herbarium is well known to contain sometimes more than one specimen for the same species, and to have been handled by hands other than those of the original owner. It is also known—more or less learned discussions to the contrary—that the *Species Plantarum* is an outright compilation based upon the sexual system in lieu of a key. Discrepancies of interpretation of the "types" in the Linnean herbarium, consequently, demand a full investigation of the *entire content* of this herbarium, and the utmost caution in the application of the synonyms. Cases arise in which an old name must be sacrificed on the altar of the goddess Typification, but the sacrifice must never be allowed without question. Linnaeus had no understanding of the interspecific limits of petty *Chamaesyce* forms, and it would be a mistake to lightly prefer his judgment—if any—to that of Boissier.

The Arnold Arboretum has a complete set of microfilms covering the Linnean herbarium, and these reproductions have been properly enlarged and made available for immediate reference. The enlargements fall short of the perspicuous details apparent in other photographs of classic material from European herbaria, but are good enough, nevertheless, for the current purposes of taxonomy.

These microfilms and their enlargements prove that the Linnean *E. maculata* rested in origin upon two specimens, as follows.

(1) The specimen seen by Boissier, and indeed very well agreeing with Fosberg's *E. supina* Raf. (op. cit. 198, fig. 2) ; the holotype of this I have studied, bringing of it a fragment, the kind gift of Prof. H. Humbert of Paris, to the herbarium of the Arnold Arboretum. This specimen (No. 630.11 in Savage's "Catalogue") is inscribed "*21. maculata*" in an handwriting which is to all appearances Linnaeus' own.

(2) The specimen, a photograph of which was handed to Wheeler as the "type," and is mistakenly reproduced by Fosberg as such (op. cit. 199, fig. 4.). This photograph is of a plant in the vicinity of the form currently understood as *E. Preslii* or *E. brasiliensis,* and is inscribed in different handwritings both "*maculata*" and "*hypericifolia.*"

I much regret that the Linnean herbarium was not open for study when I visited London at the end of 1938, and will not venture to commit myself to certain identification of, or notes about, these plants, the intangibles of which must be studied upon the actual sheets. I am safe in the belief, nevertheless, that Boissier, who knew *Euphorbia* and studied the Linnean "types,"

in choosing one fully agreeing to sound practice was not wrong in identifying *E. maculata.* In short, Wheeler and Fosberg, unknowingly despite Boissier's outspoken warning, are trying to do in 1939, 1941, and 1946 what Boissier did in 1862, and to do it against Boissier's prior understanding. This cannot be done, and on this the matter rests.

It is consequently proper to affirm the following synonymy,

> *Euphorbia maculata* L. sensu Boissier in DC. Prodr. **15**(2): 46. 1862. Syn. *E. supina* Raf.; Wheeler, Contr. Gray Herb. **127**: 76. 1939; Fosberg, Rhodora **48**: 197. 1946.

Considering the narrow scope of this note, I see no reason to complicate matters with a reference to *Chamaesyce,* which I emphatically hold to be the correct generic name for the group to which *E. maculata* and *E. Chamaesyce* belong.

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THE ROLE OF CERTAIN ENVIRONMENTAL FACTORS IN GROWTH AND REPRODUCTION OF PHOTOSIPHON BOTRYOIDES KLEBS—III. REPRODUCTION[1]

SISTER M. LAURENCE MAHER, I.H.M.

Klebs (1896) and Moewus (1933) (table 1) emphasized the important role of physical and chemical factors in stimulating reproduction of *Protosiphon*. Klebs' work, however, was probably based mostly upon studies of impure (unialgal) cultures. Pringsheim and Ondraček (1939) have been unable to confirm portions of Moewus' work.

FACTORS INFLUENCING GAMETE PRODUCTION—PHYSICAL FACTORS

A. Temperature. In order to test the effect of temperature on gamete formation, a loopful of material grown on agar was transferred to each of several depression slides or watch glasses. Both depression slides and watch glasses were kept in moist chambers in covered Petri dishes Table 14 lists data that are typical of the results obtained when the material is transferred to water. Four depression slides with thalli grown for two weeks on 0.06 per cent Bristol's solution solidified with 1 per cent agar were used for each set of temperature conditions.

Heat always acted as a stimulus to gamete formation. In unheated material, the process was slower in beginning and continued for days, particularly if the dishes were standing at a temperature below 20° C. Keeping dishes at a temperature of 28–32° C for one-half to one hour and then returning them to room temperature (18 5° C) had an effect on gamete formation similar to keeping dishes with plants immersed in liquid at a temperature a few degrees lower for a longer period. Temperatures of 28–32° C were usually more effective when they were used for one-half to two hours than when they were used for longer periods. An increase in temperature of 5–10° C above that at which the plants were grown always brings about gamete formation when plants that are at least two weeks old are immersed in water.

B. Age of Material. Material 2–12 weeks old was used for experiments on gamete formation. It was taken from cultures in which the sacs were in vegetative condition and also from cultures in which the sacs contained coenocysts, both rēd and green. The formation of gametes appeared to be

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[1] Part I of this series appeared in Bull. Torrey Club 73: 573–587; Part II in 74: 20–37. Several of the tables and figures herein cited are found in Parts I and II; the summary and list of literature cited in Part III refer to the entire series.

a little slower from red coencysts, but essentially the results were the same in so far as the age of the plants was concerned.

C. Concentration of Solution. One loopful of material from clone 6B1 grown 37 days on 0.06 per cent Bristol's solution in 1.5 per cent agar (pH 5.7) was transferred to each of four depression slides containing 0.06 per cent Bristol's solution. A similar series of depression slides with 0.25 per cent Bristol's solution was set up. Six hours later, more gametes were observed on the slides with 0.25 per cent Bristol's solution than in those with less concentrated solution. The next morning gametes were very numerous on all the slides. Two days later, however, there were more zygotes and fewer

TABLE 14. *Effect of temperature on the rate of gamete formation in clone 8B1.*

Temperature	No. hrs. required for gamete formation	Observations
18 5° C (control).	8	Gametes came out in sticky bunches.
28° C for one hour then 18.5° C.	4	Gametes from the same sac stuck together. More motile forms than in those not heated, but the same sticky appearance.
29° C for one hour then 18.5° C.	4	Some motile forms after four hours. Gametes much more numerous, after twenty four hours, than in either of the above.
29° C for four hours, then 18.5° C.	4	Gametes less numerous after four hours than in the dishes that were heated for one hour. After two days, also, gametes were less numerous than in dishes heated for one hour.
30° C for four hours, then 18.5°C.	4	Gametes very numerous after four hours. After twenty-four hours, gametes were less numerous than in the other heated dishes.

vegetative sacs on the slides with 0.06 per cent Bristol's solution. In 0.25 per cent Bristol's solution, zygotes were less numerous, and the proportion of sacs that had not formed gametes was greater than in the more dilute solution.

A loopful of vigorously growing vegetative thalli was transferred to depression slides in triplicate. The groups of depression slides contained respectively distilled water, 0.06, 0.12, 0.25, 1.0, and 2.0 per cent Bristol's solution. At the end of four and one half hours, gametes were numerous on the slides containing distilled water. They continued to be more numerous in this series than in any of the others during the first 24 hours. After 48 hours, motile gametes were no longer present. The contents of sacs that had

not formed gametes were still in vegetative condition. In 0.12 and 0.25 per cent Bristol's solution, after four and one-half hours, gametes were fewer than in distilled water. The process continued for a longer time than in the more dilute media and motile forms were still numerous after 48 hours. In 0.5 per cent Bristol's solution, no gametes had been discharged after four and one half hours, conjugating gametes were numerous after 24 hours, but there were none after 48 hours. In 1.0 per cent Bristol's solution, some of the sacs formed gametes, but they were not motile and none was liberated at any time during the period of observation. The contents of some of the larger sacs formed coenocysts. In 2.0 per cent Bristol's solution, no gametes were produced; after 24 hours, all the larger sacs and many of the smaller ones contained coenocysts.

D. Effect of Light on Gamete Formation. It has been noted many times in the course of the work that cultures on agar very frequently contained gametes on the day following their removal to a region of weaker illumination. Subsequent experiments indicate that weaker illumination stimulates gamete formation. Thalli from clone 6B1, grown for 37 days on 0.06 per cent Bristol's solution in 1.5 per cent agar, were used to test the effect of light on gamete formation. Light of three varying degrees of intensity was tested: bright light, six inches from a 300-watt water-cooled lamp, diffuse light from a north window with translucent glass, and darkness. The temperatures were similar throughout. Four depressions on slides with distilled water were employed for each light source. After four and one half hours, gametes were very numerous on the slides in diffuse light, in bright light gametes were less numerous than in either diffuse light or in darkness, and many more gametes were produced in darkness than in either of the other series. No differences were observed when experiments were begun at different times of day. Vegetative sacs of clone 7B2 transferred to liquid at 1:00 p.m. under identical conditions formed numerous gametes in four hours just as did those transferred at 9:00 a.m.

FACTORS INFLUENCING GAMETE PRODUCTION—CHEMICAL FACTORS

A. Effect of pH on Gamete Formation. The hydrogen-ion concentration at which material was previously grown apparently had no effect on the formation of gametes when the thalli were transferred to liquid. For example, material of clone 6B1 grown sixteen days on 0.06 per cent Bristol's solution in 1.5 per cent agar and with pH values of 4.1, 5.1, 6.3, 7.1, and 9.0 was transferred to series of three depression slides containing 0.06 per cent Bristol's solution at pH 4.7. After four hours at 24° C, gametes were present in all the slides. Twenty-four hours later conditions in all the dishes were the same as to motility, numbers of gametes, etc., indicating that the hydro-

gen-ion concentration of the media in which the plants were grown had no effect on rate or abundance of gamete formation.

The pH of the liquid to which the plants were transferred did, however, have a marked influence. Plants grown on 0.06 per cent Bristol's solution at pH 3.6 in 1.5 per cent agar were transferred to 0.06 per cent Bristol's solution at pH 3.6. At a temperature of 23° C gametes were present five hours later, but the harmful effect of the medium on the gametes was soon apparent. They did not show the usual prolonged motility, but rounded up at once and sank to the bottom of the slides around the sac from which they had been liberated. After ten days, there were a number of zygotes in the slides, while many partially fused cells that had not formed walls were disintegrating.

Some of the same material was transferred to depression slides with Bristol's solution at pH 5.1. Here gametes were much more numerous and motile than in the more acid solution, but there were also some disintegrating cells.

Thalli from clone 6B1 grown for seventeen days on 0.06 per cent Bristol's solution at pH 5.0 were immersed in distilled water and in 0.06 per cent Bristol's solution at pH 4.1, pH 7.0, and pH 9.2, at 24° C and at 27° C. Four depression slides were employed for each experimental condition. After four hours, gametes were present in all the slides. In each medium, they were more numerous on the slides kept at 27° C than on those at the lower temperature. Fewest gametes were present at pH 9.2 and most at pH 7.0 where they were so numerous that the medium appeared green after four hours.

B. Effect of Organic Media on Gamete Formation. Plants grown for six weeks on 0.06 per cent Bristol's solution were transferred to depression slides containing distilled water, 0.06 per cent Bristol's solution, 1, 5, and 10 per cent sucrose, and stored at 27° C. After five hours, gametes were present in all the slides. Gametes in 1 per cent sucrose were less numerous than in water or 0.06 per cent Bristol's solution, but more numerous than in the higher concentrations of sucrose. After 24 hours, gametes were more numerous in 1 per cent sucrose than in any other medium. After 48 hours, zygotes were more numerous in 1 per cent sucrose than in inorganic media, and there were no disintegrating cells on the bottom of the slides as there were in those containing distilled water. Just as in inorganic media, gametogenesis continued for longer time in the higher concentrations of organic media, and motile forms were still present in 5 and 10 per cent sucrose after 48 hours.

PRESENCE OF A PYRENOID

Presence or absence of a pyrenoid in the gamete was one of the characters used by Moewus (1935b) to distinguish the different races with which

he worked. He states that in the *B* race, a pyrenoid is present when gametes are formed in organic medium; lacking when they are formed in inorganic medium; and present in some and lacking in others when an organic substance is added to an inorganic medium.

Gametes shown in figure 39 were produced in 1 per cent sucrose. It can be seen that some have pyrenoids and others do not. The same observations were made when gametes produced in water or in 0.06 per cent Bristol's solution were stained with iodine. In the *S* race, likewise, pyrenoids were observed in some and not in others regardless of the nature of the medium. Gametes from the *M* race produced in both organic and inorganic media were stained with iodine, but no pyrenoid was ever observed. Gametes from the *N* race produced in water (fig. 15) contained darkly staining inclusions when treated with iodine and a pyrenoid was observed in some of them. Gametes from the *V* race produced in 0.06 per cent Bristol's solution with 0.5 per cent inulin also contained a number of darkly staining inclusions. Some contained pyrenoids. These observations on the pyrenoid of gametes do not confirm those of Moewus.

FACTORS INDUCING CONJUGATION

Zygotes were not observed inside the sacs except in the *V* culture (fig. 16), but they were obtained repeatedly when single sacs in a suitable stage of development were transferred singly to distilled water in depression slides. (They were continually present in all the clonal cultures.) For example, four single sacs from clone 7*B*2 were transferred separately to depression slides and kept at a temperature of 18.5° C; conjugating gametes were present the next day and four days later zygotes were observed. Similarly eight sacs from clone 8*B*2 were transferred singly to depression slides with distilled water at a temperature of 19° C. One day later, zygotes were observed on all the slides. Three sacs of 6*B*1 were transferred singly to drops of water on agar. Three days later zygotes were present in each drop. These experiments indicate that the *B* race is homothallic. Tests with single sacs from other races were not made, but the presence of zygotes in all the clonal cultures indicates that they also are homothallic.

Temperature. Zygotes were found in all the cultures where there was sufficient water for the gametes to swim. As a rule, slides that had been subjected to preliminary changes in temperature contained more zygotes than those kept at constant temperature. For purposes of comparison, counts were made of the number of zygospores on five or more fields and the average number recorded. Temperatures up to 34° C were used without inhibiting copulation. In some cases, more zygotes were produced when heat was ap-

plied for a shorter interval than when the dishes were kept at a higher temperature for a longer period (table 15).

An exact count of zygotes is impossible because they are present in all stages of development and cannot be readily distinguished from non-motile unfused gametes until they are mature (fig. 40).

TABLE 15. *Effect of temperature on zygote formation in clone 6B3.*

Temperature	Average number of zygotes per field three days later
14.5° C (control).	10
34° C for one hour, then 14.5° C.	30
34° C for one half hour, then 14.5° C.	40
30° C for one hour, then 14.5° C	20
30° C for one half hour, then 20° C.	25
30° C for one half hour, then 14.5° C.	30

Even when material was kept at constant temperature zygotes were frequently very numerous. Plants two weeks old from clone 6B1 were transferred to distilled water in four depression slides and kept at a temperature of 18.5° C; a similar series of depression slides with material from the same culture was kept at 20° C. After three days, practically all the cells present in both series of slides were zygotes. Zygotes may be very numerous also on solid media. They were extremely numerous on 1 per cent agar in Petri dishes two days after the cultures were inoculated with clone 6B1. The temperature was 23.5° C.
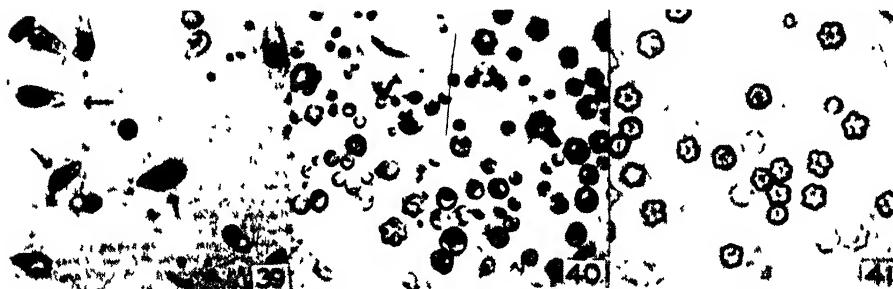
FIG. 39. Gametes from clone 2B1 24 hours after thalli were transferred to 1.0 per cent sucrose solution. × 675. The cells were stained with dilute iodine solution; arrows indicate cells showing pyrenoids. Some cells show three of the four flagella indicating that fusion of gametes has taken place. FIG. 40. Zygotes from clone 6B3 three days after thalli were transferred to depression slides with water. They were kept at 30° C for one hour and then at 20.5° C. × 350. FIG. 41. Zygospores from clone 6B1 ten days after thalli were transferred to depression slides. × 350.

Ability to copulate was not lost when the temperature was lowered. A culture of clone 7B2 was kept for 16½ hours at a temperature of 33° C. Some

thalli were then transferred to water in depression slides and kept at 6° C for four hours and then at 16° C. Another series from the same culture was kept at 16° C; in both series copulating gametes were numerous after 24 hours.

Material of the same age (two weeks) from clone 8B2, not previously treated with heat, was placed in watch glasses and exposed to a temperature of 33° C for four hours, then stored at 16° C. Copulating gametes were fairly numerous after 24 hours and zygotes were very numerous after three days.

Light. Zygotes were formed both in light and in darkness. It was not determined with certainty whether or not one was more effective than the other in this respect. In the experiment on the effect of bright light, diffuse light, and darkness described previously, zygotes appeared to be more numerous in the slides stored in bright light than they were in the others. In similar experiments, however, no significant difference in the relative numbers of zygotes was observed. Light is not essential to the formation of zygotes, inasmuch as they were observed in cultures that had been kept in total darkness for three months.

Concentration of Medium. Zygotes were produced in greater numbers in less concentrated solutions. When 0.06, 0.12, 0.25, and 0.5 per cent concentrations of Bristol's solution were used, zygotes were most numerous in 0.06 per cent Bristol's solution. When 0.06, 1.0, and 2.0 per cent concentrations of Beyerinck's solution were used, zygotes were also much less numerous in the higher concentrations.

TABLE 16. *Effect of pH on the formation of zygotes in clone 6B1*

Plants growing in 0.06 per cent Bristol's solution in 1.5 per cent agar at pH 5.0 for seventeen days were transferred to 0.06 per cent Bristol's solution with pH adjusted to the values indicated.

pH	Temperature	Number of zygotes per field two days later
Distilled water	24° C.	25
4.1	24° C.	5
4.1	27° C.	20
7.0	24° C.	12
7.0	27° C.	15
9.2	24° C.	40

High concentrations of sucrose were found less effective in stimulating the production of zygotes than 1 per cent solution. Zygotes were more numerous however in 1 per cent sucrose than in distilled water or 0.6 per cent Bristol's solution when all other conditions were identical.

Hydrogen-ion Concentration. Zygotes were very numerous both in liquid and on agar for all the pH values that were observed in this connection. They were unexpectedly numerous on agar at pH 3.6, a hydrogen-ion concentration so far from optimum that there was no growth when the solution was used in liquid form.

When sacs grown on agar were transferred to groups of four depression slides with 0.06 per cent Bristol's solution at various pH values, the number of gametes that initiated fusion but dropped to the bottom and disintegrated was greater in acid than in basic media. This may account for the higher number of zygotes observed in basic media (table 16).

The large number of fusing cells at pH 4.1 that disintegrated without forming a cell wall indicated that this solution was unfavorable for good development of gametes. Figure 41 shows zygotes from the solution at pH 9.2, ten days after the slides were prepared.

<div align="center">DISCUSSION</div>

Protosiphon botryoides is not so favorable an organism for quantitative study as such an alga as *Chlorella*. The latter possesses such advantages as more homogeneous cell size and distribution through the culture medium, both of which are absent in *Protosiphon*. The tendency of some cells of *Protosiphon* cultures to form gametes readily during the experimental period (owing, perhaps, to micro-environmental differences), while others continue to grow as vegetative individuals, renders attempts to secure quantitative data more difficult. This is further complicated by the fact that the developing plants are often crowded together in portions of the culture dishes as a result of the phototactic responses of the gametes, so that the ultimate size attained by individual cells is probably influenced by intercellular competition. In spite of these difficulties it seemed desirable to obtain quantitative data from the experiments, especially because of Moewus' rather categorical conclusions as to the influence of various environmental factors. To this end such criteria of growth were employed as mean size of 100 sacs per culture, density according to arbitrary color standards and amount of centrifugate. In the light of the difficulties mentioned above, the results obtained from such analyses are at best approximations.

The races of *Protosiphon* available for the present study were isolated from soil from New York, N. Y. (*B* race); Pennsylvania (*M* race); Virginia (*V* race); Nebraska (*N* race); and Syracuse, N. Y. (*S* race).

Cultures from the *B* and *M* races have been grown side by side at various temperatures in both organic and inorganic media for more than three years. Under the most favorable conditions, the length of sacs from the *M* race is from one-fifth to one-fourth that of the *B* race. Iyengar (1933) described

an Indian form that he calls *Protosiphon botryoides* Klebs forma *parieticola* f. nov. It was found on the walls of houses and compounds where droppings of birds and other organic matter were present during the monsoon season. He reports that plants near the bottom of the wall where conditions were more favorable had a short rhizoidal portion and resembled *Chlorococcum* and if it were not for the series of transitional forms might be mistaken for that genus. Under less favorable conditions, cells of the M race also lack rhizoidal portions and resemble *Chlorococcum*. The dimensions given by Iyengar are not significantly different from the corresponding dimensions of plants from the M race, as is apparent from the following data:

Sacs from	Length	Width	Diameter of coenocysts
Indian form	0.2 mm.	0.07 mm.	0.018–0.3 mm.
M race	0.15 mm.	0.04 mm.	0.015–0.078 mm.

In both forms, the smaller sacs frequently form one large coenocyst. Iyengar says that one gamete of a fusing pair is frequently larger than the other. In the M race some gametes were twice as large as others.

A desert form of *Protosiphon* was discovered by Nayal (1933) and called by him *Protosiphon botryoides* (Kutz.) Klebs var. *deserti*. The average size of the plants (1.25–1.6 mm. long and 0.13–0.18 mm. broad) is approximately the same as that of the B race. The difference seems to be in the gametes which are described as fusing in an end-to-end position without first becoming laterally apposed. He reports that the gametes are isogamous, but that those from the same mother-cell do not fuse, i.e. this race is heterothallic. Some of Nayal's illustrations of "compound" swarmers are very similar to forms commonly seen in cultures of the M race. He interprets them as masses of two or more swarmers which did not separate before escaping from their mother cells. He reports that some of them separate while swimming, and that others behave as asexual zoospores. Klebs (1896) also reports fusion of more than two gametes. The large and peculiarly shaped zygotes from the V race (fig. 17) appear to have been formed from more than two gametes.

The zygospore is unfavorable for cytological study (Bold 1933), but such study would doubtless shed much light on the role played by "compound" forms in the life cycle of the organism.

Except for the peculiar budding from the top of cells in the V race (fig. 13), which was not seen in the other races, the N, V, and S races are very similar to the B race.

The use of an artificial light source for growing cultures of algae produces uniform results and is used by many modern investigators (Bold 1942; Pringsheim 1946). However, cultures kept for three months in total darkness showed considerable growth. This also is in agreement with the findings

of other investigators. Beyerinck (1898) described the growth of *Chlorella*, *Scenedesmus*, and other algae in total darkness and concluded that the nutrition of such forms may be either autotrophic or saprophytic depending upon the composition of the medium. Dangeard (1933) found that cultures of *Scenedesmus acutus* maintained their green color after eight years in total darkness. Meyers (1940) used a photoelectric spectrophotometer to observe the pigments of *Chlorella* and other algae that were grown in darkness in organic media. He could detect no difference between pigments produced in darkness and those produced in light. Bristol-Roach (1928), working with *Scenedesmus*, found that the addition of sugar increased the growth in light as well as in darkness, and concluded that normal photosynthesis and assimilation of sugar may be carried on independently and simultaneously by soil algae, and that growth in the lower layers of the soil is at the expense of organic matter in the soil. According to Ruben and Kamen (1940) the chief difference between photosynthetic and non-photosynthetic reactions is the fact that the former obtain energy from light and are endothermic and the latter obtain necessary energy from exothermic reactions.

The experiments with *Protosiphon* indicate that the amount of material needed to supply energy is very small, inasmuch as the only organic material available in some liquid cultures where considerable growth occurred was the small amount apparently transferred with the thalli when the inoculations were made. On slants, there was more growth than in liquid media because of the organic matter available from the agar (Robbins 1940).

The concentration of agar in various media (table 7) markedly influences cell size as Moewus (1933) also observed. The longest sacs after 24 days growth in 0.06 per cent Bristol's solution with various concentrations of agar were produced on slants with 0.5 per cent agar. In Pringsheim's solution, however, the longest sacs occurred at an agar concentration of 0.75, 1.0, and 1.5 per cent. The average length of sacs on these three concentrations was the same. Moewus (1933) stated that 0.7 per cent agar was the optimum concentration for solidifying nutritive media for growing *Protosiphon*. Cells from liquid media tend to remain spherical (figs. 22, 33) while the same media solidified with low concentrations of agar (0.5–1.0 per cent) stimulate growth of the rhizoidal portion (fig. 23). This is undoubtedly correlated with the natural habitat of *Protosiphon* on soil, and probably is a manifestation of both positive thigmotropism and phototropism.

The composition of Pringsheim's solution differs considerably from that of Bristol's solution, and in most cases the length of the sacs was less in Pringsheim's solution (table 7), but the form of the plotted curve was the same. This is an indication that the difference in results was not due to difference in composition.

Although length of sacs is not a reliable criterion for amount of growth in liquid media, nonetheless it appears to be significant that in four of the six media listed in table 6, sacs grown in solution with a concentration of approximately 0.06 per cent of nutrient salts are longer than those grown in higher concentrations. A concentration of 0.06 per cent was accordingly used for all cultures except those used for testing the effect of concentration.

The data summarized in table 6 agree with the expectation that, inasmuch as *Protosiphon* is found so universally in soils, it must be capable of growing on nutrient materials rather widely distributed and under varying conditions. Results with Beyerinck's solution were variable. In table 6, the total yield of centrifugate from this solution was small. In the experiment designed to compare equal concentrations of different media, the yield was good. The principal difference between this and other media used is that the proportion of potassium is less. Reed (1907) states that cells of *Spirogyra* elongate but do not divide when potassium is lacking in the nutrient solution. The low final hydrogen-ion concentration of the solution no doubt has an effect on the results obtained. Hoagland (1944) emphasizes the role played by potassium in plant buffer systems.

In the three culture media used for studying the effect of different NO_3/NH_4 ionic ratios (tables 8, 9) the largest sacs were produced in the cultures with nitrate nitrogen only. In all three after 24 days growth, the smallest sacs were found in the cultures with ammonium nitrogen only. Cultures on Bristol's solution with ionic ratio 50 NO_3/50 NH_4, at the end of 24 days were in vegetative condition, indicating that they were still growing. The yield from flasks with medium in liquid form was also higher than from any of the other liquid media. The presence of well developed sacs (fig. 31) was a further indication that this medium was favorable to good growth.

In Pringsheim's solution, factors other than nitrogen source had a bearing on the results: in Bristol's and Detmer's solutions, most of the sacs in cultures with nitrate nitrogen contained coenocysts and increase in thallus size had ceased; in Pringsheim's solution with nitrate nitrogen, on the contrary, the sacs were still in vegetative condition and consequently capable of growing longer. In Pringsheim's solution with ionic ratio 50 NO_3/50 NH_4, a precipitate was present that may have removed needed ions from the solution, thus inhibiting growth.

The hydrogen-ion concentration of Detmer's solutions with the lower NO_3/NH_4 ratios was already too low for good growth when the solutions were inoculated, and it dropped still lower, indicating that with the absorption of ammonium ions, OH ions were also being removed from solution. The appearance of cells from liquid cultures indicated that the solutions were unsuitable for good growth. Trelease and Trelease (1933, 1935) emphasized the advantages of employing solutions in which the proportions of nitrate

and ammonium nitrogen are physiologically balanced so that under the influence of absorption and excretion of substances by the plant, hydrogen-ion concentration would tend to remain constant.

Pringsheim (1946) states that delicate algae which lose their fresh color in solutions containing nitrate or ammonium salts only, or in those containing ammonium nitrate, may be reared successfully in media with nitrogen supplied by two different salts. Chu (1942) concluded that planktonic algae with few exceptions grow equally well in media with nitrate and those with ammonium salts as long as the nitrogen concentration is within the optimum range.

For about seventy-five years, it was assumed that only ten elements were indispensable for the growth of the higher plants. Refinement of methods in recent years has made it possible to prove that other elements in addition to the traditional ten are needed. Hoagland (1944) states that the necessity for boron and manganese has been demonstrated by many investigators and accepted by all. Evidence for the need of copper and zinc is less extensive, partly in the case of copper at least, because of the difficulty of excluding the small amount of the element in the form of impurities. Both elements are included in the list of essential element by Hoagland (1944).

Lipman (1940) notes the dire need for many more investigations of the chemical elements needed by microscopic plants. Studies that prove elements essential for higher plants do not necessarily prove that the same elements are needed by lower plants. Pringsheim (1946) states that the fact that much more is known concerning nutrient solutions for higher plants shows that there is still a wide and promising field of investigation with respect to the algae. Bold (1942) found that micrometabolic elements are undoubtedly important in the nutrition of algae and recommended that they be included in all culture solutions in spite of the possibility of their introduction as impurities in other salts.

A minor element solution containing the four elements mentioned above has been used by Craig and Trelease (1937) for the culture of *Chlorella.* It was found that the addition of very small amounts (approximately 0.007 ppm. of B, 0.0003 ppm. of Cu, 0.01 ppm. of Zn, and 0.02 ppm. of Mn) to the undiluted culture medium produced results that could be readily seen and measured.

In 1939, Arnon and Stout showed that tomato plants grown in purified solutions with eleven essential elements developed deficiency symptoms that could be prevented by adding one part of molybdenum to 100,000,000 parts of nutrient solution. Steinberg (1937) demonstrated that molybdenum is required by *Aspergillus niger* to a greater degree when nitrate is the source of nitrogen than when ammonium or organic nitrogen is supplied. In the present study it was found that 2 ml. of molybdic acid solution added to

0.25 per cent Bristol's solution (1 to 250,000,000 parts of nutrient solution) gave an increase in growth that could be seen and measured.

Moewus (1935a) reported that he obtained good growth of *Protosiphon* in solid media with a pH range of 4.5–9.5. The present experiments in general confirm this, but at the same time indicate that *Protosiphon* is more tolerant of media with low pH values when the latter are solidified with agar. The increase in growth observed in this and in other experiments when media is solidified with agar may indicate an adsorption of certain ions by the agar, but it seems more probable that the explanation given by a number of investigators in growth of fungi applies here. Fries (1938), Leonian and Lilly (1940), Robbins (1940, 1941), and others observed that the amount of growth was greater in a medium solidified with agar than in the same medium used in liquid form. They attribute the difference to presence of growth substances in the agar and in some instances demonstrated specific growth substances responsible for the results.

On acid media, sacs and coenocysts were formed more quickly than on media that were more nearly neutral, but the plants did not become so large. This is in accord with the conclusions of Artari (1913), who found that *Chlamydomonas* grows faster at first in an acid medium, but that ultimately the amount of the growth was the same when a more alkaline medium was used. Pringsheim (1926a) recommends neutral or slightly alkaline media as most favorable for most algae. In the experiments with *Protosiphon* it was . found that media with pH values ranging from 4.5 to 8.5 gave good results in both liquid and solid media. On solid media, good growth was secured throughout the range used (pH 3–9.5). In liquid there was no growth in cultures with pH values lower than 3.6.

Moewus (1935a) used cultures with pH values from 4.5 to 9.5, but Pringsheim and Andraček (1939) obtained growth of *Protosiphon* only between pH 5.5 and 8.0. They report that plants were decolorized in basic solution and remained green for some time in acid solution with a pH value lower than 5.5, but did not grow. The present study confirms Moewus' results in this connection.

Growth in media with various hydrogen-ion concentrations evokes considerable variation in form. In acid media sacs elongate very readily in either liquid or solid substrata, and are characterized by a club-shaped green portion and a colorless rhizoidal portion. In the more alkaline solutions, the cells remain spherical in liquid and in some cases, sacs growing inside a spherical mother cell have the appearance of colonial forms (fig. 33).

The calcium requirement of *Protosiphon* was investigated in connection with the preparation of solutions with different pH values. In some of the alkaline solutions the calcium compound caused the formation of a precipitate. In some cases, the presence of a precipitate did not interfere with

growth, but in others it appeared to inhibit it. It was found that good growth could be obtained when calcium was omitted from the culture medium. This does not necessarily mean that no calcium was present, however. Magnesium compounds were included in all the formulae used. Mast and Pace (1939) analyzed by means of spectrograms a number of the purest magnesium salts that could be obtained and found that calcium was always present as an impurity. Chu (1942) states that the Ca requirement for algae is lower when the Mg requirement is higher. .

It is generally held that calcium is necessary for growth and reproduction of all organisms except fungi, and some flagellates and algae. Pringsheim (1926b) used sodium oxalate to precipitate calcium from media used to grow algae. He divided the algae studied into forms that did not need calcium and were not inhibited in their growth by sodium oxalate in the concentrations used and those that did need calcium and upon which sodium oxalate had a toxic effect. He made no attempt to prove total absence of calcium and states that calcium traces can be assimilated in the presence of sodium oxalate. He states (1946) that calcium should always be provided in media used to culture algae, but mostly in very low concentrations because of its tendency to precipitate phosphates and iron unless the solution is so definitely acid that it would be harmful to most species.

When *Protosiphon* was grown in 0.06 per cent Bristol's solution (table 10) with the concentrations of sodium oxalate used by Pringsheim (1926b) it was found that there was better growth in the solutions with sodium oxalate than in the controls both with and without calcium. This increase in growth obscured the effect of lack of calcium.

Mann (1932) found that the concentrations of sodium oxalate used by Pringsheim increased the growth of *Aspergillus niger* and concluded that the stimulating effect might be due either to the effect of some impurity in the sodium oxalate or to ability of the fungus to utilize carbon from the oxalate. In view of the stimulation of growth obtained when additional carbon in the form of carbohydrates is added to the medium used to culture *Protosiphon* it seems reasonable to assume that this organism also is capable of utilizing carbon from sodium oxalate.

It has been clearly demonstrated by Chodat (1913) and more recently by Hall and Schoenborn (1938) that chlorophyll-bearing algae are stimulated by the addition of organic substances to the culture medium. The addition of a number of different carbohydrates to the media used for growing *Protosiphon* resulted in an increase in the length of sacs grown on agar and in the amount of material centrifuged from flasks with liquid media. Inulin was found to be most effective in promoting growth and xylose least so. Nearly twice as much material was centrifuged from flasks with 0.5 per cent inulin as from those with 0.5 per cent dextrose. Very good growth was

obtained also with fructose. This is to be expected, inasmuch as inulin yields two molecules of fructose on hydrolysis.

Matruchot and Molliard (1902), Artari (1906), Kufferath (1921), and others have compared the effect of different carbohydrates on various types of algae. Artari (1913) discussed the conflicting reports regarding ability to grow in the dark, position of pyrenoids, and other reactions to different media, and concluded that it was possible that cultures from the same species and having the same morphology might belong to different physiological races.

Extensive experiments were not performed on the effect of proteins on the growth of *Protosiphon;* it was found, however, that very good growth was secured with beef extract and with peptone used either alone or with 0.06 per cent Bristol's solution. Pringsheim (1946) states that the usual peptones, prepared from muscle, contain sufficient mineral substances so that further additions are usually unnecessary.

The importance of the relative proportions of carbohydrates and of organic compounds has long been recognized. Artari (1906) reported that the value of different nitrogen sources depends to a great extent on the presence of glucose. He concluded that ammonium nitrate was a better source of nitrogen for *Chlorella communis* than potassium nitrate when glucose is present. Kraus and Kraybill (1918) emphasized the importance of the relative proportions of carbohydrates and organic nitrogenous compounds in the carbohydrate-nitrogen ratio in their work on tomato plants.

Vischer (1926) and Wall (1939, 1940) subscribe to a theory that deficiency of potassium affects the synthesis of protein from the simpler compounds. Hoagland (1944) states that evidence of the immediate necessity of potassium for the condensation of sugar units to form starch or other polysaccharides is insufficient, but the effects on carbohydrate metabolism and synthesis when plants are grown for a long time under potassium deficiency show that potassium plays some part in the process.

Steinberg (1939) found that admixture of carbon compounds incapable of assimilation when they were the sole carbon sources markedly increased their assimilability. He stated that *Aspergillus niger* was capable of maximum growth with inorganic nitrogen and sucrose. He interpreted the result as mutual supplementation of compounds deficient in essential molecular configurations.

These references and innumerable others that might be mentioned emphasize the complexity of problems dealing with metabolism.

Klebs (1896) and Moewus (1933, 1935a) (see tables 1 and 2) both investigated the influence of environmental factors on gamete formation. Klebs' work was based on unialgal cultures, but Moewus claims to have used pure cultures. The experiments summarized under "factors influencing gamete

production'' represent a preliminary attempt to confirm the results of these investigators. As noted by them, change in temperature has a marked effect on the rate of formation. The time required varied from four to twenty-four hours. In material transferred to liquid and then exposed for periods up to six hours to temperatures a few degrees higher than that at which the cultures had been growing, gamete formation is much accelerated, and begins sooner than in material treated identically except for change in temperature. Four or five hours treatment at a temperature of 28° C evoked lively formation of gametes from material in the coenocyst stage; at 18.5° C, from eight to nine hours were required. The present observations that short periods of increased temperature decrease the time required for gamete formation correspond with the results reported by Klebs (1896), but the temperatures here used are higher than those he found effective. He states that 24–26° C is optimum and that within these limits, two and one-half hours is sufficient time for gamete formation. At 8° C and below according to Klebs, more than nine hours are required. Moewus (1933), on the other hand, reports that at all temperatures between 7° and 28° C gametes were formed in four to five hours apparently at the same rate. Cultures one to eight weeks old grown on 0.7 per cent agar were used by Moewus. The cells were transferred from agar to tap water, distilled water, and to Kolkwitz' and other solutions. He reports that no gametes were formed at 5° C and under, or at 30° C and over, but that their formation is as rapid at 7° as at 21° C, or at Klebs' optimum of 23–26° C when the cells were previously cultivated at room temperature. No confirmation of Moewus' (1933) conclusions in this regard have been obtained in the present investigations.

When material from *Protosiphon* cultures from two to twelve weeks old was transferred to liquid for gamete formation, differences in the length of time required for the process could not be attributed to the age of the material. Gametes were produced from vegetative thalli seventeen days old in the same time required for gamete formation from red coenocysts twelve weeks old. At room temperature, the rate of gamete formation in older cultures appeared to be a little slower, but the differences in time required for gamete formation to begin in any of the experimnts with material of different ages at the same temperature were not great. The results are in agreement with those of Moewus (1933) to the extent that cultures of different ages and from different media require approximately the same amount of time for gamete formation to begin when they are put into the same medium and kept at the same temperature. Klebs (1896) found that gamete formation from red coenocysts was usually slower and required 24 hours at room temperature. In the present study, 24 hours were sometimes required when the temperature was below 18° C, but the same amount of time was required by cultures two weeks old under the same conditions.

The observation that concentration of the medium has a marked bearing on the process of gamete formation is at variance with Moewus' (1933) report but supports Klebs (1896). Gamete formation was more rapid but of shorter duration in 0.06 per cent Bristol's solution than in higher concentrations. It continued for 24 hours in 0.06 and in 0.5 per cent Bristol's solution and for 48 hours in concentrations of 0.12 and 0.25 per cent solutions. In 1.0 and 2.0 per cent conentrations of Bristol's and Beyerinck's solutions, most of the sacs had formed coenocysts after 24 hours and few if any gametes were produced in liquids with such concentrations. Klebs (1896) and Moewus (1933) reported gamete formation in 1.0 per cent nutrient solution. Klebs stagted that gamete formation was slower as the concentration of the solution increased, but Moewus (1933) includes media with a concentration of 1.0 per cent among the factors that have no effect on the rate of gamete formation.

Klebs' statement that lack of light is a stimulus for gamete formation was confirmed in the experiment with effect of light on gamete formation. Slides kept in darkness contained more gametes after four and one-half hours than those in dim light or in bright light. Moewus (1933) stated that there was no difference in rate of gamete formation in light and in darkness.

Klebs (1896) and Moewus (1933) also investigated the effect of chemicals in stimulating gamete formation and reached various conclusions summarized in tables 1 and 2. In the present study, when thalli grown on media with various pH values were transferred to 0.06 per cent Bristol's solution (pH 4.7), no difference in the rate of gamete production could be observed. When thalli grown at a given pH value were transferred to 0.06 per cent Bristol's solution with various pH values, the effect of different degrees of acidity was marked. In 0.06 per cent Bristol's solution with pH 3.6, the period of motility of the gametes was short. They appeared to round up immediately and could often be seen on the bottom of the depression slides around the sac from which they were produced. In acid media, the period of active gamete formation was shorter than in media with a reaction nearer to neutral or in alkaline media.

Moewus (1935a) reported that races of *Protosiphon* with phenotypic sex determination (homothallic) produce more + than − gametes when acid solution (pH 4.5) is used for culture and gamete formation and that there are more − than + gametes after culture and gamete formation in alkaline medium. At pH 7.0, + residual gametes are present in half the dishes and − residual gametes in the other half of the dishes. He denominated cells reacting in this way as "phenotypically dioecious" and concluded that sex was not determined during gamete formation, but during the 48 hours following gamete formation.

The classification of the *B* race (obtained by Moewus from Bold, and also

used in these studies) by Moewus (1935b) as dioecious with genotypic sex determination, and his explanation that a mutation may have taken place have already been mentioned. Bold (1933) reported that he was not certain whether or not meiosis occurs when the zygote germinates, but that by analogy with the conditions known to exist in some other forms it may do so. As authority that meiosis takes place when the zygote germinates, Moewus (1935b) cites Kniep (1928). Kniep, however, states that we cannot answer finally if *Protosiphon* has genotypic sex determination, but that it is *presumed* that such a difference is necessary for copulation, and that a reduction division *probably* takes place at or before germination.

Moewus (1935b) assumed that since reduction division occurs at the germination of the zygote, the developing plants are haploid. When the germinating zygotes reached a certain size (mostly 8-nucleate), he transferred them to the surface of agar in Petri dishes. The surface of the dishes was covered with water, and they were kept in darkness at a temperature of 28–30° C. At that temperature, according to Moewus, copulation did not take place, and the darkness kept the gametes from collecting phototactically. He transferred these individual plants developing in such cultures, and assumed they were clonal.

Bold (1933), however, points out that the uninucleate segments of thalli or coenocysts formed by cleavage are not definitive gametes, but they undergo one or two bipartitions before they become gametes. Moewus (1935b) refers to this and states that because of the nuclear divisions referred to by Bold (1933), the number of swarmers that goes out from a cell is always a multiple of two. He states that in three out of sixty-five hundred developing zygotes less than eight haploid clones were obtained because one or more gametes failed to develop. In all other cases, he states that eight haploid clones were obtained and cytological investigation always showed eight nuclei in the developing zygotes. From germinating zygotes of the *B* race he obtained four + and four − clones. He agrees with Bold (1933) that the nuclei divide before gametes are formed, but does not explain why the division of eight nuclei results in the production of eight instead of sixteen or even thirty-two gametes.

In the present study, this method of securing clones was unsuccessful, partly because it was found that a temperature of 28° C' stimulates copulation. Klebs (1896) also states that a constant temperature of 26–27° C during the last phase of gametogenesis inhibits the ability to conjugate, and reports that ability to conjugate is lost when the gametes are brought to a lower temperature. Neither of these statements could be confirmed, for zygotes were formed in temperatures up to 34° C. Slides with swarmers were frequently subjected to an increase in temperature and then returned

to a lower one without any loss in ability to conjugate. Change in temperature on the contrary appears to be a stimulus for conjugation.

In 1.0 or 2.0 per cent inorganic medium, the contents of the sacs changed to coenocysts and few if any either of swarmers or zygotes were seen. In 1.0 per cent sucrose zygotes were more numerous than in distilled water or in 0.06 per cent Bristol's solution. These results are in agreement with those reported by Klebs (1896), but not with those of Moewus (1935b) who states that copulation takes place in 1.0 per cent Kolkwitz' solution and also in a concentration twenty times higher. ·

No clear-cut relation between hydrogen-ion concentration and number of zygotes was observed (table 16). With all other conditions the same, change in temperature appeared to be a more important factor. On agar slants, zyogtes were more numerous when the temperature was above 22° C, regardless of hydrogen-ion concentration. ·

According to Moewus (1935b) + and – gametes should be formed in equal numbers by thalli from the B race. If the sex determination were phenotypic, according to the same author, both high temperature and acidity should combine to give an increase in the number of + residual gametes and correspondingly less conjugation. In alkaline medium there would be more – residual gametes.

No evidence of sexual dimorphism was found in the present study. Material from the B race was used for all the experiments with environmental factors, but zygotes were seen in all the clonal cultures of all the races studied, all of which are therefore monoecious or homothallic. In all the slides with thalli immersed in liquid and also on agar, the formation of zygotes was such a continuous process that it was impossible to determine whether the smaller cells were rounded up unfused gametes or zygotes that had not yet acquired the characteristic star-shaped wall. Pringsheim and Ondraček (1939) likewise found that the process of zygote formation was a continuous one, and were unable to secure residual gametes even when they removed motile unfused ones to distilled water in separate dishes.

Carter (1926) noted that gametes of *Protosiphon* sometimes fuse within the mother cell without being released. Klebs (1896) stated that gametes from the same mother do not, as a rule, copulate, but that zygotes are present occasionally in sacs containing coenocysts. Zygotes from the V race are shown enclosed in a sac in figure 16. Zygotes appear to be formed more readily in this than in some of the other races. They were often observed on depression slides to which single thalli had been transferred. In some cases zygotes were not obtained either because the temperature was not conducive to copulation or because the sacs were not in a suitable condition.

Observation of cultures under different environmental conditions provided some evidence that gametes develop directly into new thalli when all

the conditions are favorable and that some stimulus such as a change in temperature or hydrogen-ion concentration is required to initiate conjugation.

Bold (1933) has described the germination of a zygote into a thallus, and there is no doubt that this is the usual method of development. It seems possible, although the process was not observed, that gametes may sometimes be produced from zygospores (fig. 41). Empty zygospore walls were observed in a number of cultures. On the slide from which figure 41 was photographed, there were in addition to the empty zygospore walls rows of small new zygospores that were being formed throughout the 38 days that this set of slides was kept under observation. In none of the empty cells was there any evidence of degenerating cell contents.

SUMMARY
(of the entire work)

A study of the effect of controlled environmental factors on the growth of certain clones of *Protosiphon botryoides* has revealed the following:

1. Cultures grown in artificial light are more successful than those grown in natural light. Ten hours daily illumination from a 300-watt water-cooled bulb seems to be optimum.

2. In a growth period of 24 days, sacs with an average length of 0.94 mm. were secured on 0.06 per cent Bristol's solution modified by substituting 0.3 gram of K_2HPO_4 for the same weight of K_2HPO_4 in the formula, thus obtaining a pH value of approximately 6.3. Minor elements solution was added to the medium which was congealed with 0.5 per cent agar.

3. Agar concentrations of from 0.5 to 1.5 per cent are optimum for normal growth. Larger sacs were developed on solid than in liquid media. After 24 days, the longest sacs (0.94 mm.) occurred in 0.06 Bristol's solution with 0.5 per cent agar. After 40 days, the longest sacs (1.3 mm.) developed on 0.06 per cent Bristol's solution with 1.5 per cent agar.

4. *Protosiphon* grows readily in a number of different media commonly used for the cultivation of algae. In inorganic media, a concentration of 0.06 per cent by weight was more satisfactory than either higher or lower ones.

5. The largest sacs were formed at a continuous temperature of 17° C.

6. The addition of 0.25 per cent inulin to inorganic media increased the amount of growth more than does the addition of any other carbohydrate. The addition of 0.25 per cent glucose increases the amount of growth in light and effects good growth in darkness.

7. Pyrenoids are present in some gametes from the *B* race and not in others, whether they are produced in inorganic or organic or both types of nutrients.

8. In acid media (pH 3.4–6.9) sacs are formed more luxuriantly than in basic media. In the more alkaline media used (pH 8.4–9.5) most sacs remained spherical in shape and were not differentiated into the club-shaped green portion and the clear rhizoidal portion in the substrate that is characteristic of plants growing in acid media or in nature.

9. Sacs with lobes and branches are of much more common occurrence in acid than they are in basic media.

10. On solid media, there was good growth in media with pH values ranging from 3.0 to 9.5. In liquid media, there was growth from pH 4.1 to 9.6. Media with pH values from 6.0 to 7.0 are optimum.

11. Nearly all solutions in which *Protosiphon* was grown changed toward a neutral hydrogen-ion concentration whether they were originally acid or alkaline in reaction.

12. The amount of calcium present as impurities in the salts used to make up culture media is sufficient for good growth of *Protosiphon*. Growth is excellent in solutions containing sodium oxalate, probably because of utilization of the carbon rather than because of absence of calcium.

13. Increase in temperature hastens the formation of gametes. Exposure of cultures grown at 17–20° C to temperatures of 24–31° C for periods of one-half to four hours results in the production of gametes in from four to six hours.

14. Zygotes were present in all the clonal cultures studied. The clones available are therefore homothallic. Zygotes are produced in temperatures as high as 34° C.

15. Zygotes are more numerous in inorganic culture media at 0.06 per cent concentration than at concentrations of 1.0 or 2.0 per cent.

16. No sexual dimorphism was found, and all the swarmers appear to be capable either of developing immediately into vegetative plants, or of fusing to form zygotes.

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TORREYA

Plant Hormones[1]

P. W. Zimmerman

I have chosen to talk to you about a comparatively new subject, "plant hormones."

To show the growing interest in the subject, I recall that in 1935 when the A.A.A.S. met in St. Louis only five papers were presented on the subject of growth substances. At the meeting just concluded in Boston more than 25 per cent of all papers in Plant Physiology were on some phase of plant growth-regulating substances. In the American Society for Horticultural Science meetings 11 of 12 papers presented on Sunday morning involved plant hormones. In the section on Floriculture and Ornamental Horticulture 5 of 11 papers concerned plant hormones. Several papers presented in the General Section were on this subject. The line of approach varied from highly theoretical to factual information and practical applications.

In 1935 much attention was given to curvatures of stems, epinasty of leaves, and responses of the *Avena* coleoptile. This year very little was heard of these responses, although they still remain as standard methods of testing chemicals for physiological activity. No one method can be used for all purposes. Had we clung to one method much of what we now know about growth-regulating substances would have been lost.

In the spring of 1935 there were only a few well recognized active substances—indoleacetic, auxins A and B being the most talked of. It is now doubtful if auxins A and B actually exist, but there are literally hundreds of other substances to take their place.

There is still some feeling and controversy over the meaning of terms and definitions of physiologically active substances. Regardless of the original intention for the meaning of the word "auxin," it is now generally considered synonymous with "plant hormones" and "plant growth-regulating substances." The term "growth substance" probably should be reserved for vitamin-like substances essential for growth of lower organisms. '

Owing to the varied types of responses induced by plant hormones, the subject drew the attention of botanists, horticulturists, and chemists. Professors at universities found the field a fertile one for graduate students and assigned many projects for theses on this subject. Publications started pouring out and have increased since 1935 to the point where it is difficult to keep up on the subject.

No one person or group of persons could ever have discovered all the facts that are now known about plant growth hormones. Through fear, suspicion, interest, or curiosity one worker picks up where another leaves off and advances our sphere of knowledge. However, no man's word is law. All claims must be tested and proved before they are acceptable in science.

[1] Address of the retiring President of the Torrey Botanical Club presented at the annual meeting on January 7, 1947.

Interest in the subject of plant hormones from the standpoint of fundamental science is still uppermost in the minds of many workers. It is increasingly evident, however, that an attack of practical problems in this field also contributes to fundamental science. It may be said that when practical problems are properly studied, the results of the investigation contribute to both science and practice. In the beginning most of the responses induced by plant hormones were looked upon as laboratory curiosities. It was not long, however, until most of these curiosities led the way to practical applications.

News writers pounced upon curiosities to arouse the interest of the public. They gave much publicity to our so-called upside-down plants, meaning that when tops were removed and plant hormones applied, roots grew on the wrong end of the plant. Leaves, flower petals, peduncles, and petioles all produced roots, making odd-looking plants. Out of these monstrosities, however, came practical methods for propagation of plants. Difficultly propagated species were caused to form roots on cuttings. Easily rooted species were accelerated. Amateurs and practical growers alike now depend upon hormones for propagation of plants.

Ethylene gas induced curvatures on plants and caused leaves to fall. Apples give off ethylene. Therefore, apples placed in storage with rose plants caused the leaves to fall. Ethylene gas and perhaps other chemicals can and will be used by growers to defoliate their plants before they are put into permanent storage. Defoliation is often desirable to prevent loss of moisture and spoilage.

Contrasted to defoliation with ethylene gas is the fact that naphthaleneacetic acid prevents abscission of leaves. This discovery soon led to the practical application, now well known, that naphthaleneacetic acid prevents pre-harvest drop of apples.

The following established practices are now well founded and have resulted from plant hormone research:

Prevention of pre-harvest drop of apples with naphthaleneacetic acid.

Increased fruit set and induced seedless tomatoes with numerous hormone-like substances.

Inhibition of buds to prevent potatoes from sprouting by the use of vapors from esters of naphthaleneacetic acid.

Inhibition of fruit tree buds to prevent loss through late frost, though the method is not yet perfected.

Regulation of flowering of pineapples involving acetylene, naphthaleneacetic acid, naphthoxyacetic acid, and substituted phenoxy compounds. In this way a crop can be staggered to facilitate harvest and canning operations.

Defoliation of plants with ethylene, acetylene, propylene gases, and other chemical means.

Thinning of fruit, especially apple, with hormone sprays, bringing about better quality of fruit and causing biennial-bearing trees to become annual-bearing.

Inhibition and killing of plant tissue, leading to the use of highly active plant hormones as herbicides, for which 2,4-D has been highly publicized.

One of the best illustrations of growth-regulation of plants is the modification of the pattern of new organs growing under the influence of applied hormone-like

chemicals. Substituted phenoxy, benzoic, and naphthoxy acids are particularly effective for this purpose. Mature organs do not change their shape, but all new growth which occurs under the new chemical influence is modified. This leads to the assumption that normal leaf patterns are determined by the natural chemical influences within the plant. Under new and stronger chemical influences the leaves and other organs assume a new pattern. So far no practical application for this odd response had been found. However, we should not be surprised if one crops up, since nearly all facts eventually find some practical use. At least, induced formative effects afford a subject for speculation, to which botanists are so well adapted.

To date no hormone-like chemicals or any one chemical alone has been found to stimulate growth of the entire plant in the same sense as is recognized for complete fertilizers. Some claims for such have been published, but they have not stood the test of time.

Flowers of solanaceous plants last abnormally long after being treated with hormone-like substances which induce parthenocarpic development. This fact is very suggestive and gives immediately the idea that the life of cut flowers can be prolonged. Unfortunately, to date these phenomena seem to apply only to intact plants. No effective chemical means for increasing the life of cut flowers have been discovered.

From our experience with root-inducing substances, modification of leaves, etc., it appears evident that all organs of the plant are under some regulating influences, probably of a chemical nature. It would seem therefore that we should find *flower-inducing substances* and *shoot-inducing substances*. While there have been various claims for shoot-inducing substances, this is not a reality in the same sense that we have root-inducing substances. It is logical, however, to assume that such chemicals do exist in nature and eventually may be found. Once they are located, if they ever should be, progress and spectacular results with such substances should be as outstanding as with well known hormone-like substances.

If we may predict something for the future of plant hormones, we should list some of the following:

Induction of shoots where they do not normally appear, thus facilitating the propagation of plant parts without buds. For example, internodes do not normally produce adventitious buds. A shoot-inducing substance applied to an internode should cause new shoots to arise where desired to make possible propagation of budless parts or to improve the shape and appearance of intact plants.

Flower-forming substances which are thought to exist in nature may be isolated, identified, and used as a common tool. If this becomes a reality it should be possible to force long-day types to flower during the short days or short-day types to flower during long days. In short, it should be possible to induce flowering of plants at will and to force flowers at unusual places. Imagine plants with flowers on internodes, on leaves, and even on roots.

At the present time there appears to be considerable variation in time of ripening of fruit. Under the influence of chemicals, the time of ripening should fit into our needs. Certain varieties of English holly growing on the western coast, though very prolific, fail to ripen berries in time for the Christmas trade. It is conceivable that with the proper treatment this variety can be caused to ripen its fruit so that it will be useful at Christmas time. During the past season it has been shown that apples

treated with certain hormone-like chemicals ripen prematurely. In a lesser degree this has been noted for tomatoes. The tomato flavors are not affected under the influence of the chemical. Apples, however, change flavor and consistency, but the very fact that modifications in time of ripening have been demonstrated, offers encouragement for practical methods applicable to all fruits.

Since it has been demonstrated that fruit buds can be delayed through treatment with growth substances, one is led to the assumption that fruiting of tropical species and flowering of plants in general can be staggered to extend throughout the entire season. Mangoes, for example, flower and ripen fruit at very definite periods of the year. During the rest of the year they are not available as food. Since this is an important tropical food, it would be desirable to extend it throughout the season. This should become a reality by the proper hormone applications to growing buds. The idea is particularly applicable to tropical plants because the temperature and other growing conditions would not limit the time of fruiting. We should, however, be able also to stagger flowering of spring shrubs in the north so that we could enjoy them over longer periods.

From the fundamental research angle many workers are trying to find how hormones work. Much emphasis is being placed on enzymatic activities, precursors, and ultimate active substances. There still remains in the minds of many people the idea that the so-called natural plant hormones are necessary for growth. In contrast to this, however, there are those who believe the so-called hormone-like substances are by-products of metabolism and that they play no important rôle in natural growth. These questions remain to be answered in the future, but they will in all probability be answered. Solutions for problems of this sort will contribute much toward our understanding of botanical sciences.

PROCEEDINGS OF THE CLUB

Minutes of the Meeting of October 16, 1946. Dr. Zimmerman opened the regular meeting of the Torrey Botanical Club at 3: 30 p.m. at The New York Botanical Garden. Twenty members and friends were present. The minutes of the previous meeting were approved as corrected. Two annual members and 19 associate members were unanimously elected.

It was recommended that the memorial to the late Dr. R. A. Harper be published in TORREYA. (See p. 87 of the January issue of the BULLETIN.)

There was no further business to transact and the meeting was turned over to Dr. Sophia Satina who spoke on: ''Chimeras among Plants and How They Arise.''

The meeting adjourned at 4: 45 p.m., and refreshments were served by members of the staff of the Garden.

Respectfully submitted,
LIBERO AJELLO
Recording Secretary

Minutes of the Meeting of November 6, 1946. The regular evening meeting of the Torrey Botanical Club was called to order by Dr. Zimmerman at 8: 15 p.m. at Hunter College. Sixty members and guests were present. The minutes of the previous meeting were approved as read. Four associate members and one annual member were unanimously elected.

Several proposed amendments to the Club's constitution were read and the members voted that the amendments be referred to the Council for further action.

Since there was no further business, the meeting was turned over to Dr. John A.

Small who gave an enlightening lecture on: ''The Pine Barrens of New Jersey,'' illustrated with many fine Kodachromes.

The meeting adjourned at 9:40 p.m. and was followed by refreshments served by members of the Hunter College Biology Department.

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Respectfully submitted,

LIBERO AJELLO

Recording Secretary
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Minutes of the Meeting of November 20, 1946. The regular afternoon meeting of the Torrey Botanical Club was opened by Dr. Zimmerman at 3:30 p.m. in the members' room of The New York Botanical Garden. Thirty members and guests were present. The minutes of the previous meeting were approved as read.

Dr. Zimmerman asked the members to give thought to the proposed compilation and publication of a cumulative index to the first 75 volumes of the BULLETIN of the Torrey Botanical Club and to present any suggestions that they may have on the subject.

Dr. Simpson brought to the attention of the members an error in the opening time of the December 18th meeting as listed in the *Bulletin* of the New York Academy of Sciences. The correct time for the meeting is Wednesday afternoon, December 18th, at 3:30 p.m. at The New York Botanical Garden.

No further business was transacted and the meeting was turned over to Dr. W. C. Price, who spoke on ''The Isolation, Identification and Characterization of Southern Bean Mosaic Virus.''

The meeting was adjourned at 4:50 p.m. and was followed by refreshments served by members of the Staff of The New York Botanical Garden.

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Respectfully submitted,

LIBERO AJELLO,

Recording Secretary
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Minutes of the Meeting of December 3, 1946. The regular evening meeting of the Torrey Botanical Club was opened by Dr. Zimmerman at 8:10 p.m. at Columbia University. Twenty members and guests were present. The minutes of the previous meeting were approved as read. The names of two prospective annual members were presented to the Club and both were unanimously elected to membership.

No further business was transacted and the meeting was turned over to Dr. M. F. Buell, who spoke on ''The Ecology of the Carolina Bays.'' The speaker's abstract follows:

There are four principal hypotheses concerning the origin of the Carolina Bays:—the meteorite hypothesis of Melton and Schriever, the hypothesis of complex origin by Douglas Johnson, the lagoon hypothesis of C. W. Cooke, and the biological hypothesis of Chapman Grant. The plant ecologist, particularly through paleoecological studies, can contribute knowledge of the bays that may have considerable value in deciding the validity of these various hypotheses. Reconstruction of vegetation recorded by pollen grains preserved in the bottom sediments, in other words the vegetation existing during the early development of the lakes which occupied the depressions, is particularly important. This includes both bog vegetation and the vegetation of the surrounding upland. The bottom sediments of one bog have been studied. They contain pollen representing boreal species with a corresponding absence of southern species. This suggests a colder climate than that which now prevails in the region of the bays. In fact, it suggests that this particular bay may have been formed in late Pleistocene time. Further studies on the bays should reveal whether or not they were all formed at about the same time.

The meeting was adjourned at 9:35 p.m. and was followed by refreshments served by members of the Columbia University Botany Department.

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Respectfully submitted,

LIBERO AJELLO,

Recording Secretary
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Minutes of the Meeting of December 18, 1946. The regular afternoon meeting of the Torrey Botanical Club was opened by Dr. Zimmerman at 3: 40 p.m. at The New York Botanical Garden. Twenty members and guests were present. The minutes of the previous meeting were approved as read.

Dr. Seaver moved that the Club publish a cumulative index to the first 75 volumes of the BULLETIN of the Torrey Botanical Club, as a memorial to Dr. and Mrs. Britton. The motion was seconded and carried unanimously by the members.

No further business was transacted and the meeting was turned over to Dr. Ray Dawson, who spoke on ''Corn and Pellagra—A Botanical Approach to a Problem in Human Nutrition.''

The meeting was adjourned at 4: 25 p.m. and was followed by refreshments served by members of the staff of The New York Botanical Garden.

> Respectfully submitted,
> LIBERO AJELLO,
> *Recording Secretary*

Minutes of the Annual Meeting, January 7, 1947. The annual dinner meeting of the Torrey Botanical Club was held on the evening of January 7th at the Men's Faculty Club of Columbia University. There were 80 members and friends present. Following the dinner, the business meeting was called to order by Dr. Zimmerman at 7: 30 p.m. The minutes of the previous meeting were read and approved. Sixteen annual members and five associate members were elected to membership in the Club.

The following officers and chairmen of standing committees made reports which are attached to the minutes:

> Dr. J. A. Small, *Chairman of the Field Committee*
> Dr. H. W. Rickett, *Editor*
> Dr. A. M. Hanson, *Business Manager*
> Dr. J. L. S. Simpson, *Corresponding Secretary*
> Dr. E. H. Fulling, *Treasurer*
> Dr. M. Levine, *Chairman of the Membership Committee*
> Dr. A. H Graves, *Chairman of the Special Committee on Fungi*
> Mr. L. Ajello, *Recording Secretary*

Dr. P. W. Zimmerman, as retiring President, gave a stimulating address on ''Plant Hormones,'' which is printed elsewhere in this issue of the BULLETIN.

After this talk, Dr. E. B. Matzke announced the results of the mail ballot for officers for 1947 as follows:

President—Dr. George H. Shull
1st Vice-President—Mr. Rutherford Platt
2nd Vice-President—Dr. Norma E. Pfeiffer
Corresponding Secretary—Dr. Jennie L. S. Simpson
Recording Secretary—Mr. Libero Ajello
Treasurer—Dr. E. H. Fulling
Editor—Dr. Harold W. Rickett
Bibliographer—Mrs. Lazella Schwarten
Business Manager—Dr. Harold H. Clum
Members of the Council—Dr. W. H. Camp, Miss Hester M. Rusk, Dr. Michael Levine, Dr. John S. Karling
Delegate to the Council of the N. Y. Acad. of Sciences—Dr. Edwin B. Matzke
Representatives on the Council of the A. A. A. S.—Dr. Michael Levine, Dr. George S. Avery
Representative on the Board of Managers of The New York Botanical Garden—Dr. Fred J. Seaver

Dr. Karling moved that the officers elected by ballot be approved. The motion was seconded and carried unanimously.

The meeting was then turned over to Dr. George H. Shull who spoke a few words of greeting to the Club.

The meeting was adjourned at 9: 00 p.m.

Respectfully submitted,
LIBERO AJELLO,
Recording Secretary

FIELD TRIP REPORTS

AUGUST 4. SINGAC, N. J. Undaunted by an overcast sky, which later cleared, ten intrepid Torreites and guests embarked in canoes on the Passaic River. At Two Bridges the left fork of the river was chosen for exploration. This branch meanders tortuously for many miles. There was time, however, to reach only a distance of 5 or 6 miles as the crow flies, for our botanical explorers made frequent stops and side trips into coves which penetrated the low meadows.

From the botanical viewpoint the trip was moderately interesting. The banks of the Passaic were fringed with common deciduous trees: several species of *Quercus*, *Betula nigra* plentifully, more *Acer saccharinum* (Silver Maple) than *A. rubrum*, occasionally *Fraxinus pensylvanica*, *Tilia americana*, *Salix nigra*, *Ulmus americana*, *Liquidambar*, *Platanus*, *Fagus*, *Carpinus*, and other genera. The shrubs, *Cornus amomum*, *C. racemosa*, *Cephalanthus*, and *Leucothoe racemosa* were also noted. Naturally, there were abundant festoons of Poison Ivy. The shores of the Passaic were rich with the Tall Indian Rice, *Zizania aquatica*, which was common throughout and sometimes invaded quite far into the river. At the time of this visit it littered the waters with the fallen staminate flowers. Other shore species noted growing on the mud near the water line were *Pontederia cordata*, *Nymphaea advena*, *Sagittaria latifolia*, *Alisma subcordatum*, *Saururus cernus*, *Sium suave*, *Gratiola neglecta*, and *Lindernia dubia* var. *inundata*.

Lindernia (*Ilysanthes*) and *Gratiola* have a superficial similarity, but can be told apart easily with some casual examination. *Lindernia* is glabrous, whereas *Gratiola neglecta* is glandular puberulent above; the calyx in *Lindernia* is distinctly 5-parted, whereas that of *Gratiola* seems to have 7 unequal parts because of the two bractlets which subtend the calyx-lobes. *Rumex mexicana* was quite abundant throughout the entire stretch of the trip. *Lysimachia* (*Steironema*) *hybrida* was seen several times. For the nomenclature of this species see Fernald, "The identity of *Lysimachia lanceolata*" (Rhodora 39: 438–442. 1937). Fernald here claims that Handel-Mazzetti shows that the characters relied upon to keep American *Steironema* apart break down in some Chinese species of *Lysimachia*, and that *Steironema* cannot be treated as a different genus. Fernald also points out that *L. lanceolata* is a southern plant, whereas the northern species is *L. hybrida*.

Polygonum robustius was not yet in flower, while *P. punctatum* was fully so, its slender racemes erect. These two species, in their different periods of flowering, as well as in other respects, appear as distinct entities, notwithstanding their synonymous disposition in Gray's Manual, 7th ed. *P. hydropiper* var. *projectum*, in its nodding racemes, larger red stem and broader leaves, was seen to differ from *P. punctatum* even at a distance. One of the most showy flowers was *P. pensylvanicum* (the common form with leaves glabrous beneath, named var. *laevigatum* by Fernald). Their tall bright rosy-red racemes often made a splendid back-drop for the bank's margin. Occasionally *P. lapathifolium* was noticed nearby. It was, the form with nodose joints and pale, more slender, drooping racemes, and it looked like an unfortunate relative of the former species. Adding color to the floral display, usually in the more open places, were *Asclepias incarnata*, *Spirea latifolia*, *S. tomentosa*, *Stachys hispida*, and *Vernonia*. In a meadow, where the group stepped to explore on foot, were noted *Iris prismatica*, *Pycnanthemum muticum*, *Scutellaria integrifolia*, *Polygala sanguinea*, *Oenothera tetragona*, and *Galium tinctorium*. Concerning the last plant mentioned, Hiroshi Hara (Rhodora 41: 388. 1939) claims that *G. tinctorium* is part of a circumpolar species differentiated into 3 geographical varieties or subspecies and names ours *G. trifidum* subsp. *tinctorium*.

The aquatic flora was also generally the common type. Several species of *Potamogeton*, tons of *Zosterella* (*Heteranthera*) *dubia* and *Vallisneria americana*, frequent ex-

amples of *Ceratophyllum*, and occasional *Castalia odorata* were seen. Of particular interest was the Thong-leaved Sagittaria, with its long slender phyllodia up to 1 meter long and 10 mm. wide and small floating flowers. This plant is named *S. lorata* in the "Illustrated Flora" and *S. subulata* var. *gracillima* in Gray's "New Manual of Botany," Fassett's "A Manual of Aquatic Plants" and Muenscher's "Aquatic Plants of the United States." It is well illustrated in both Britton & Brown's "Illustrated Flora" and Fassett's "Manual." As observed on the Passaic, the plant has all the appearances of a distinct species.

Homeward bound, on the way to Little Falls, the comparison of *Centaurea nigra* and *C. jacea* arrested the group's attention somewhat. It was noted that while *C. nigra* was already past blooming, *C. jacea* was at its flowering peak. These species belong to a section of *Centaurea* which is badly understood. Our observation indicated that these two, at least, behave like distinct species.

Two suggestions are made for botany students concerning the plants seen on this trip: tha. a critical field study be undertaken of the local *Centaureas*, particularly of the forms or varieties of *C. nigra*, *C. jacea*, and *C. vochinensis*, and that a detailed investigation be conducted on the identity of the Thong-leaved Sagittaria. A repetition of the trip by the Torrey Botanical Club is recommended for the future.—JOSEPH MONACHINO, Leader.

AUGUST 31–SEPTEMBER 2. BOTANICAL SURVEY and check of guidebook material between Culvers Gap and Harding Lake, N. J. A list of species was prepared for the record. Attendance 6. Leader, L. E. Hand.

SEPTEMBER 7–8. MT. EVERETT, MASS. The striking summit, locally known as "The Dome," with its scrub vegetation and rock plants was studied. A pond and a woodland with their contrasting flora were likewise examined. A small group made a flying trip to Bartholomew's Cobble, an area much desired for permanent preservation by the Trustees of Public Reservations. No list was kept but the Gray Herbarium has been instrumental in listing plants of the Cobble, of which we have copy. It is hoped that someone will offer a trip primarily to the Cobble soon. Attendance 21. Leaders, Rutherford Platt and Harold N. Moldenke.

SEPTEMBER 8. INWOOD PARK, N. Y. "The various species of trees and shrubs were seen and listed and a striking specimen of *Triosteum aurantiacum*, the Horse Gentian, with clusters of orange fruit, was seen; the small species of Wild Geranium was common." Attendance 18. Leader, Arthur H. Graves.

SEPTEMBER 14–15. FORKED RIVER, N. J. Five different walks probed the interesting countryside, and bayside. As usual here, additions were sought to the present list of recorded plants. These lists from 1944 and 1945 are now available in mimeographed form upon request to the field chairman. Most of the famous pine barrens novelties were shown to those making the visit for the first time. A good opportunity to learn distinguishing characteristics of asters, goldenrods, and Eupatoriums was afforded. Attendance 33, Leaders, Hollis Koster and L. E. Hand.

SEPTEMBER 15. MAPLE GRANGE VICINITY, SUSSEX CO., N. J. This was another Apppalachian Trail survey trip and added many new names to the list since it was the first visit to limestone country and also extended into non-calcareous areas. The list is available from the files of the field chairman but it has not been mimeographed. Attendance 5. Leader, G. G. Nearing.

SEPTEMBER 22. MILL NECK, L. I. The group enjoyed an interesting walk through forests, fields, salt and fresh water habitats. One individual specialized in a search for rusts while others paid more attention to the higher plants and passing birds. Attendance 16. Leader, Farida A. Wiley.

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COMPILED BY

LAZELLA SCHWARTEN

WITH THE COLLABORATION OF THE EDITOR OF THE TAXONOMIC INDEX

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BERNARD OGILVIE DODGE

Fred J. Seaver

Of all the living ex-presidents of The Torrey Botanical Club probably none has been more active in the affairs of the organization than Dr. B. O. Dodge who, with the loyal cooperation of his good wife, has held about every position which the Club afforded.

He was Secretary-Treasurer at the time when that title covered a multitude of duties, such as recording the minutes; collecting money and keeping records of all receipts and expenditures; negotiating contracts with the printers; writing letters; and, last but not least, delving into the depths of the subcellar at Columbia University, ferreting out from the dust-covered stock and debris back numbers of the Bulletin, and shipping them to every part of the world. In that day the Secretary-Treasurer was "Jack of all jobs" in connection with the Club. For several years he was editor of the BULLETIN and for many years he collaborated with Mrs. Dodge who was the official Bibliographer. In fact there is scarcely an activity of the Club in which the Dodges have not been concerned. For these reasons it has been thought appropriate to dedicate the present volume to Bernard Ogilvie Dodge.

The writer's first encounter with Dr. Dodge was in the fall of 1909 when the latter entered Columbia University as a graduate student, and reported to the New York Botanical Garden for research in the fungi; in this group he had acquired an interest while engaged in high school work in Wisconsin, and he maintained this interest later in the University of Wisconsin. He was assigned to work on the Ascobolaceae, a family of the Discomycetes, with the expectation that he would carry on a taxonomic study of the family. He soon became interested in the reproductive processes of these fungi; this was the beginning of his work on sex in the fungi, in which field he has made his chief contribution to science. One of his early papers was "Methods of culture and the morphology of the archicarp in certain species of Ascobolaceae" (Bull. Torrey Club **39**: 139–197. *pl. 10–15.* 1912). In this group he described a new species, *Ascobolus magnificus.* It was in connection with this work that he discovered the effect of heat in inducing germination of the ascospores of *Ascobolus,* a process which was later found applicable to the germination of the spores of other fungi, especially *Neurospora.* He continued his researches in Columbia University until 1920, working also with the plant rusts which took him into the field of pathology.

In 1920 he went to Washington as Plant Pathologist in the United States

Department of Agriculture where, although a Plant Pathologist by vocation, he continued his investigation on sex in the fungi and extended it to other groups. In his well known work on pink bread mold (*Neurospora*) he was a pioneer in the hybridizing of fungi and systematically observing the results of such crosses. Through this work he became one of the world's most outstanding mycogeneticists. His work on *Neurospora* is too well known to require here more than a passing mention.

In 1928 he accepted the position of Plant Pathologist at The New York Botanical Garden and began his work on May 1 of that year; he officially retired at the end of 1946. During his official connection with the Garden he has contributed much toward the improvement of the health of the plants of that institution, especially in the control of black spot of roses. He discovered the cause and control of a disease of the much cultivated *Pachysandra*. The disease is caused by the fungus *Pseudonectria pachysandricola*, a species described by Dr. Dodge and not previously known to science.

A scientist never really retires, he merely throws off some of his routine duties in order to devote himself more exclusively to his hobby; and Dr. Dodge is no exception to the rule. He may still be seen daily in his laboratory and will doubtless continue his researches with unabated zeal.

His book *Diseases and pests of ornamental plants*, prepared in collaboration with Dr. H. W. Rickett, is in constant use by pathologists and is the only work of its kind devoted exclusively to diseases of ornamental plants.

GROWTH CURVES OF ORIENTAL TOBACCO AND THEIR SIGNIFICANCE[1]

FREDERICK A. WOLF

From time immemorial biologists have been concerned with problems of growth. Consequently a voluminous literature on growth, including observations on both animals and plants, has accumulated. In attempting to interpret this literature and to seek an understanding of the underlying principles involved, many controversial issues have been brought to light. Judging from perusal of representative botanical investigations it would appear that all too little which is basic is yet well established. This conclusion confirms that of Glaser (1938) who stated that "When reduced to absolute dryness, a fair sample of the literature on organic growth yields a residue in which a few non-volatile platitudes are embedded in a voluminous matrix of quantitative fact."

In his recent book, "Growth and Form," Thompson (1945) evaluates the present status of knowledge of growth as it relates both to animals and to plants. It appears that present-day concepts regarding growth in plants, including both woody and herbaceous species, date from the work of Sachs (1887). His observations show that the growth of broad bean, *Vicia faba* L., begins at a slow rate but as the process continues it does so at an increasingly rapid rate until it reaches a maximum; then growth progressively decreases and eventually comes to a stop. This sequence of events may be portrayed graphically by an "S-shaped" or sigmoidal curve, now generally recognized as a common pattern among Nature's mechanisms ("mecanisme commun aux phenomenes dispartes"). Furthermore Sachs' measurements of the growth rate of the shoot-axis of *Phaseolus multiflorus* Willd. and of dahlia, if graphed, yield curves similar to that of broad bean.

Among other annual species in which growth rates have been studied are *Vallisneria spiralis* L., by Bennett (1880), maize, by Stefanowska (1904), Lehenbauer (1914), and Pearl and Surface (1915), and sunflower, by Reed and Holland (1919). It has been concluded that the precise form of the logistic or growth curve for each of these species, or, indeed, for any other species or particular individual is determined by two basic variables, one germinal and the other environmental, which interact during the develop-

mental period. As is generally appreciated, the former is constituted of a complex of internal factors and the latter of interdependent external factors.

The writer has been concerned for several years with problems of growth in connection with the production of oriental types of tobacco (Wolf & Jones 1944, Bentley & Wolf 1945, Wolf 1946). From experience gained during the course of this work, it has become increasingly apparent that a better understanding than is extant is required regarding growth of the tobacco plant, if improvement in quality and quantity of the product grown is to be secured. On the basis of published results with tobacco (Avery 1934, Morgan & Street 1935, Petrie, Watson & Ward 1939, Petrie & Arthur 1943), and with other plant species, one might logically assume that there is little likelihod of achieving this purpose or that little additional knowledge would be acquired by studying the rate of growth of tobacco. Nevertheless additional observations on growth of this crop plant have been made and the data thus secured together with their interpretation are presented herewith.

MATERIALS AND METHODS

The varieties of oriental types of tobacco used in these studies included Izmir (Smyrna), Broussa and Dere (Samsoun), Stanimaka (Cavalla), and Yaka (Xanthi). They were grown in a field at the Tobacco Experiment Station, Oxford, N. C. The cultural practices employed, including fertilization, spacing of plants, and tillage, were such as are known to be favorable for satisfactory growth. Two methods of sampling were employed. In one method, increase in height of selected plants was determined by a series of successive measurements extending over a period of about two months. At the same time the rate of expansion of leaves was followed by measuring the length and greatest width. A ratio between length-width and area had been predetermined, and on this basis leaf area was calculated, using the product of length and width as one factor in the calculation.

In the other method paired groups of ten plants each were selected and marked for analysis. Replications were used so far as time permitted. All plants from one member of the pair were permitted to grow unmolested until the seed heads were well formed. By that time the leaves along the lower portion of the stems had become dry and others above them were senescent. Other leaves, properly primed,[2] from near-by plants therefore were substituted in the analyses for these dry and overmature ones.

The leaves from all plants of the other member of the pair were primed as they matured and their inflorescences were removed as soon as the buds appeared.

In making the analyses, the plants were uprooted and brought into the

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[2] Priming is a term used to designate a method of harvesting tobacco by picking the leaves when they are in best condition for curing.

laboratory where the leaves were removed in sequence, weighed immediately, and their lengths and widths were determined. Weights and lengths of internodes were determined after the stems had been cut into segments. The average weight of the leaves at each node and that of corresponding internodes was computed.

<div align="center">RESULTS</div>

The growth form of plants of oriental varieties of tobacco should first be discussed. Their unbranched stems have the general shape of a gradually tapering cylinder, being largest in diameter near the soil level. The internodes tend to become progressively longer from the basal ones upward to the middle of the stalk and gradually shorter thereafter. An occasional plant is found having short internodes occurring between long ones, but from average measurements of corresponding internodes of a series of plants such inequalities are not so apparent. Internode length, moreover, is correlated directly with weight (mass), to such a degree that the internodes of greatest weight are produced at the median stalk region, being progressively less heavy both upward and downward from this median position.

Similarly leaf size progressively increases upward from the base of the stem, those of maximum size occurring near the median stalk position. Thence the leaves become smaller upward toward the stem tip with occasional deviations from this pattern.

Data bearing on the relation between internode weight (mass) and internode position for 4 oriental varieties are shown graphically in figure 1.

It is apparent that the jagged curves in figure 1 are all of the same type, resembling distribution or population curves. Irregularities and variations in shape from ideal curves are ascribed to the small number of plants (10–25) used, and to fluctuations in growth rate induced by differences in weather during the developmental period. Broussa and Stanimaka, both equally tall kinds, are especially to be noted and compared because the stalks of the former are sturdy and of the latter slender.

An important feature relating to the distribution of mass along the stalk which is not fully apparent in figure 1 becomes obvious, however, if the data are recalculated on the basis of division of the stalk into three regions, lower, median, and upper, each region having the same number of internodes. If this is done, it will be found that the weight of the median region is approximately equal to the combined weights of the other two regions.

In the writer's opinion there is another way, better than that employed in figure 1, to show the distribution of substance produced along the stem-axis, and to show that its rate of augmentation, expressed as "per internode," proceeds both in an orderly fashion and at different rates in different regions. This is to use "increment of growth" as the unit. Data for the variety Izmir, graphed thus, are presented in figure 2. These plants grew for a

WEIGHT (MASS)

120

110

100

90

80

70

60

50

40

30

20

10

BROUSSA

YAKA

STANIMAKA

DERE

5   10   15   20   25   30

INTERNODE POSITION

I
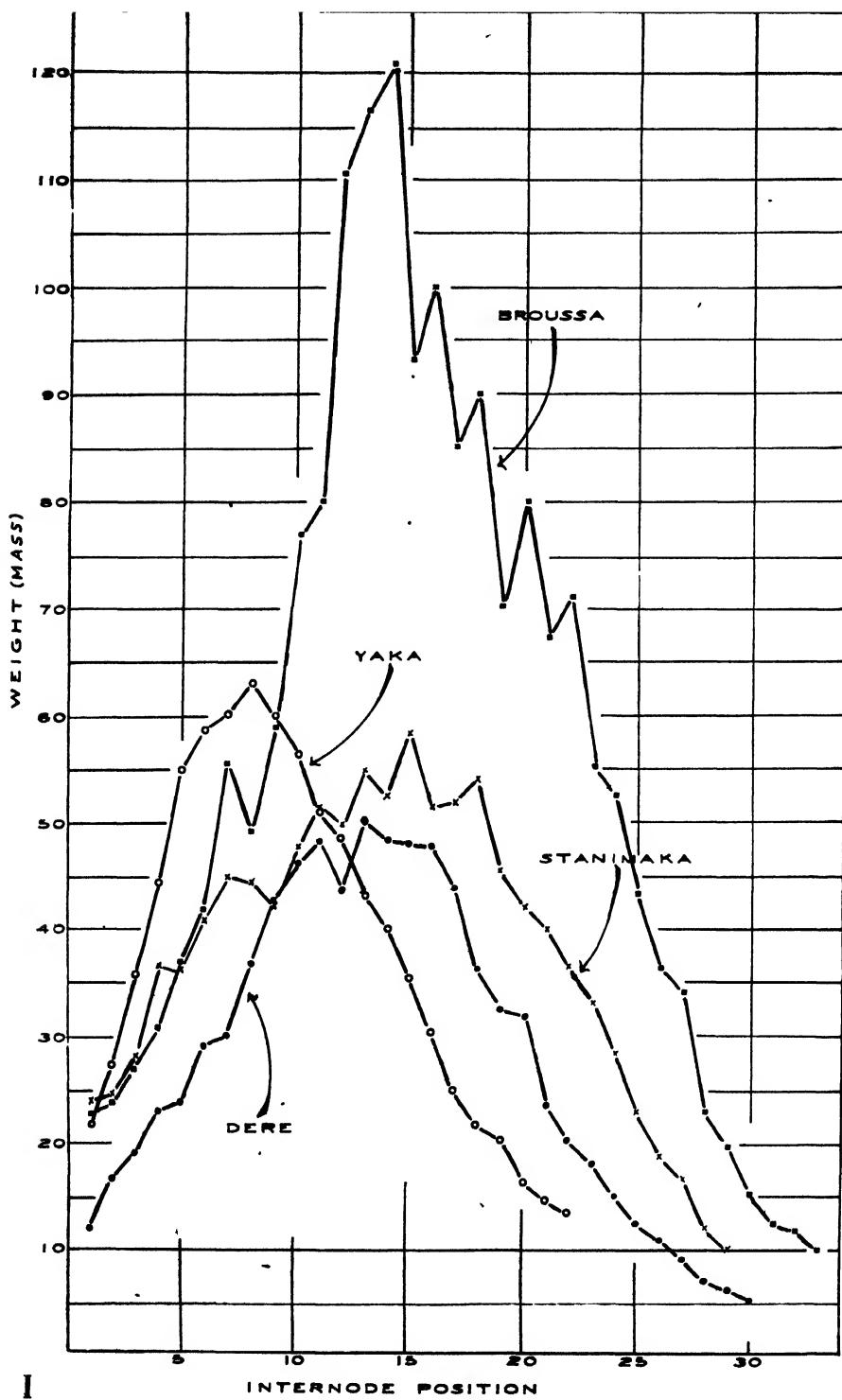
period of 86 days after transplantation. The "intervals" herein could with equal propriety be expressed as "per days," so long as it is appreciated that the elapsed time for the development of substance for any internode was not accomplished during an average of about 3 days but rather about 3 weeks. The weights shown are the combined weights of 10 plants, internode weights being combined with that of the corresponding leaf. For each succeeding internode in ascending order the weight of substance produced was added seriatim to that of the previous total. By so doing, the green weight of stem and leaf substance of approximately 20 grams had increased to more than 900 grams by the time the thirty-first segment had matured. Such progressive increases. when graphed, give a typical sigmoidal growth curve (fig. 2) in which change in rate of growth at different intervals is portrayed by change in slope of the curve.

TABLE 1. *Rate of expansion of Izmir tobacco leaves at different stalk positions*

Lower leaves			Median leaves			Upper leaves		
Date	Average leaf area, cm.^{2}	Percentage of mature size	Date	Average leaf area, cm.^{2}	Percentage of mature size	Date	Average leaf area, cm.^{2}	Percentage of mature size
June 7	9.04	8.8	June 27	13.86	12.2	July 18	8.61	18.1
" 10	19.28	18.9	July 1	40.65	35.9	" 23	30.75	64.7
" 13	40.80	40.1	" 4	71.60	63.2	" 25	37.20	78.3
" 17	81.40	80.0	" 8	101.20	89.4	" 29	42.30	89.0
" 20	94.10	92.4	" 11	108.20	95.6	Aug. 1	44.80	94.3
" 24	97.70	95.9	" 15	111.20	98.2	" 5	46.70	98.3
" 27	99.30	97.5	" 18	114.20	100.0	" 8	47.50	100.0
July 1	101.30	99.5						
" 4	101.80	100.0						

A growth curve of very similar form (fig. 3) was secured by use of other plants from which to plot, according to the usual procedure, progressive change in height against elapsed time.

A series of successive measurements was made to determine the growth rate of leaves of Izmir tobacco. These measurements involved 15 leaves from each of three stalk positions, the basal, median, and upper regions. The data from these measurements are assembled in table 1.

It is apparent from table 1 that the development of the leaf from the time that it is sufficiently large to measure while attached, until it is fully expanded requires a period of approximately 3 weeks, i.e. duration of the growth period is quite constant regardless of leaf position. It must be remem-

Explanation of figure 1

Growth curves of stems of 4 varieties of oriental tobacco, in which internode green weight is plotted against position of internode.

2

3
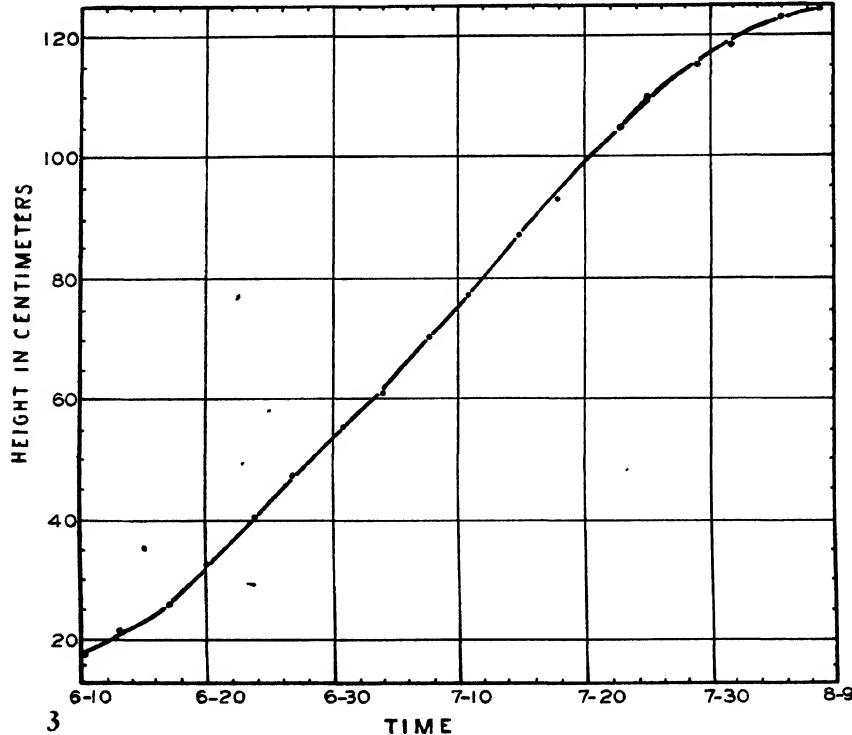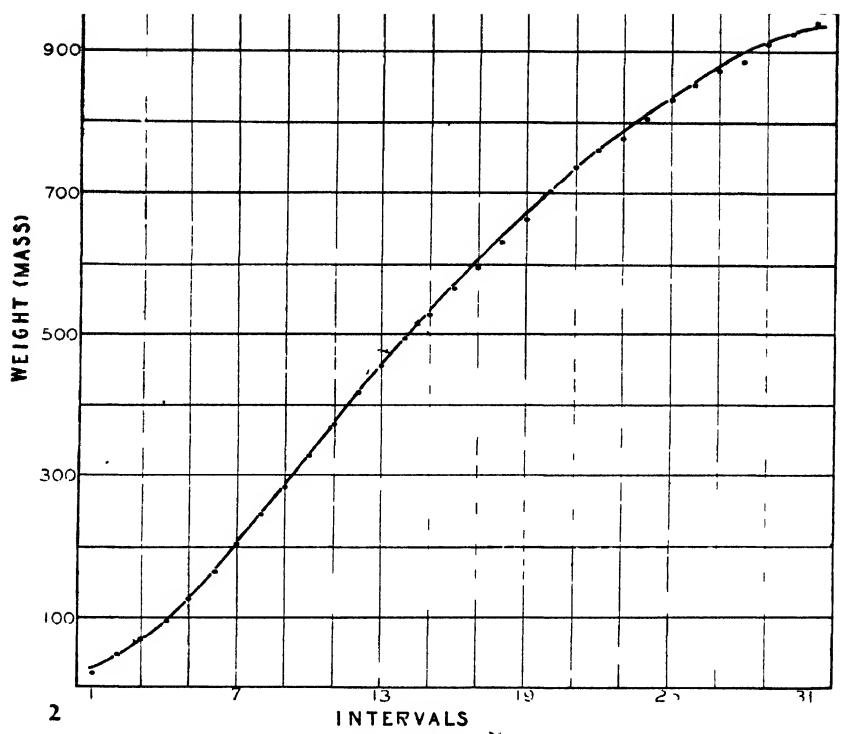
bered, however, that leaves are not prime, hence are not ready to harvest as soon as they are fully expanded. But the leaves in the median position are the largest, hence their absolute growth rate, called "intensity of growth" by Berthold (1929), is greatest. These data in table 1, however, fail to show the relative rate of growth of leaves borne at different stalk positions. By relative
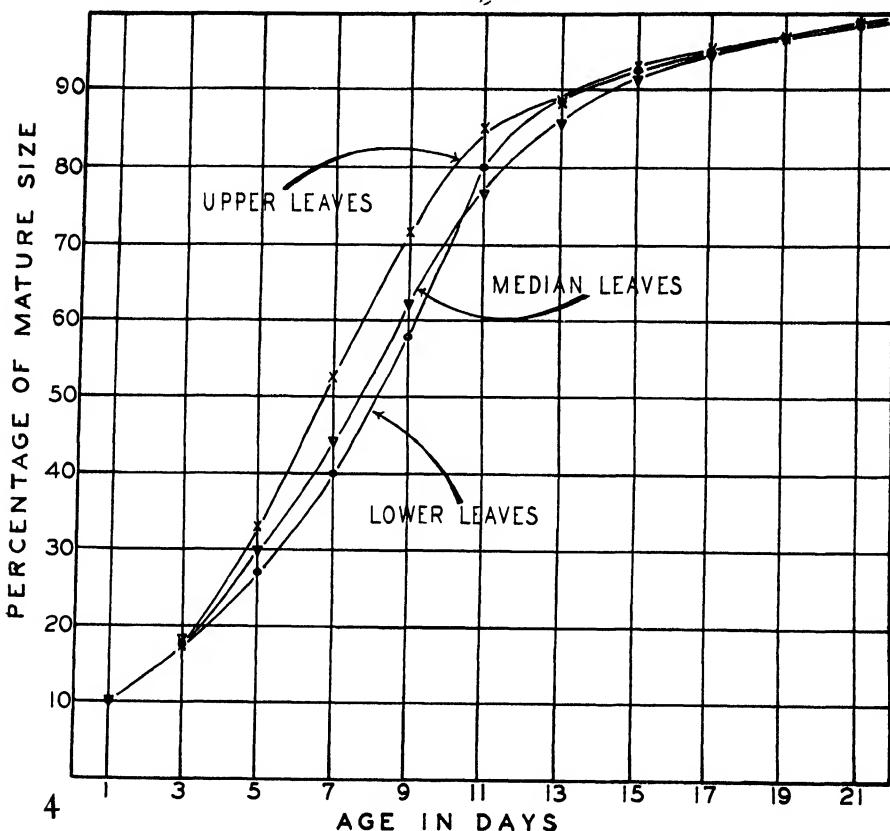
FIG. 4. Sigmoidal growth curves (calculated) showing similarity in rate and duration of expansion of leaves of Izmir tobacco at three stalk positions.

rate is meant herein the percentage daily expansion based on final size. Consequently relative rates of growth were calculated from the observed rates, with the results shown in figure 4.

When the data are presented in this way it is apparent that the leaves at

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Explanation of figures 2, 3

FIG. 2. Sigmoidal growth curve of Izmir tobacco. Combined weight of substance produced (internode and leaf) is plotted against interval (either position or time). The weight of each successive internode in ascending order augments that of combined substance at a lower level. FIG. 3. Sigmoidal growth curve of Izmir tobacco in which height is one ordinate and elapsed time is the other. Note the resemblance to figure 2.

PLANTS PRIMED AND TOPPED

PLANTS UNMOLESTED

WEIGHT (MASS)

INTERNODE AND LEAF POSITION

5

PLANTS PRIMED AND TOPPED

PLANTS UNMOLESTED

WEIGHT (MASS)

LEAF POSITION

6
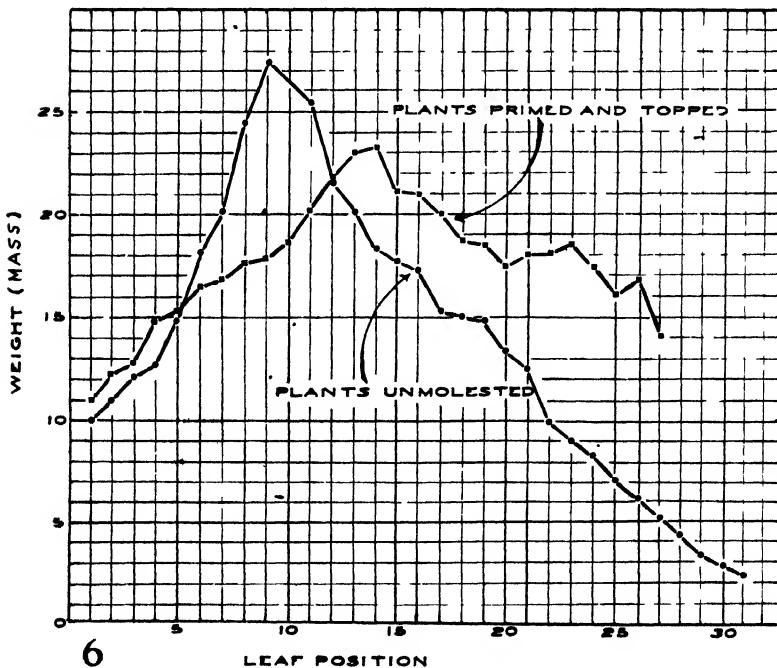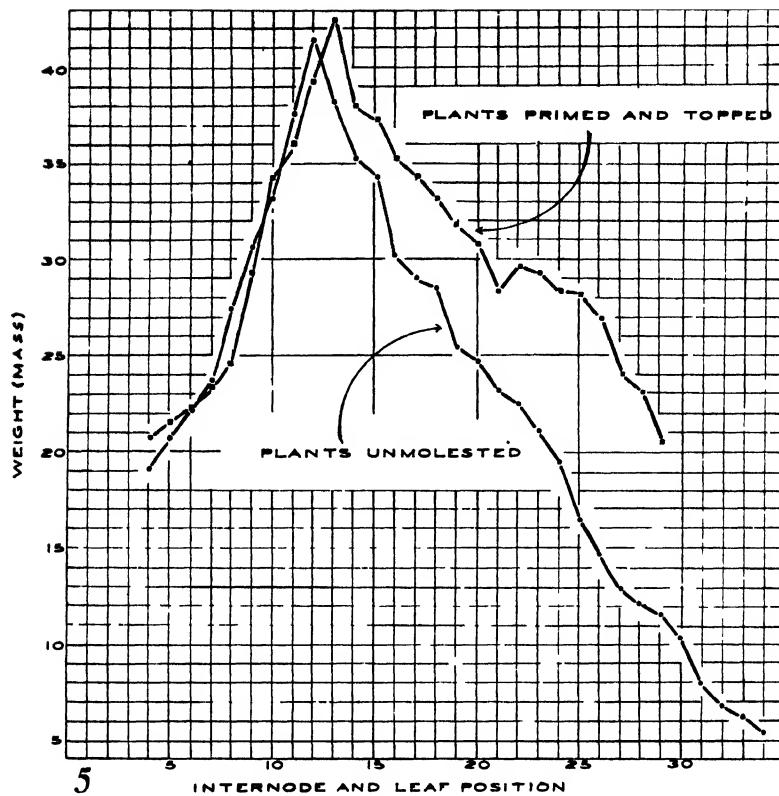
each stalk position exhibit strikingly similar growth rates. The minor differences shown may be assumed to be attributable to differences in weather prevailing during their expansion.

In quest of a correlated cause for change in rate of growth, comparative measurements were made of two groups of plants of the Izmir variety. In one group all plants were topped as soon as the floral-axis appeared, and their leaves were primed as they matured. The plants of the other group remained unmolested. Averages of the combined weights of internode and corresponding leaf plotted against position in ascending order for these two groups of plants may be compared in figure 5.

The most noteworthy feature presented in figure 5 is the increased green weight of substance produced at the upper portion of the topped, primed plants. It has long been known that the uppermost leaves become larger in response to topping and there is coincidentally an increase in stem substance. To isolate "increase in leaf weight" from "combined weight of internode and leaf," the weights of leaves alone from the same sets of plants were similarly graphed in figure 6. It should be noted that as a result of topping the point of inflection of the growth curve is shifted farther upward toward the stem tip.

In figure 6 the influence of priming is not separated from that of topping but it may be presumed by comparison with figure 5 that the greater effect is induced by topping. At any rate green weight of leaf substance seems to be modified to a greater degree than that of stem substance. Furthermore it may be concluded that rate of growth along the upper portion of the stem-axis is accomplished under the dominance of internal factors arising from the inflorescence.

INTERPRETIVE DISCUSSION

As anticipated, a gradient of growth rate from one stalk position to an adjacent one in the development of the tobacco plant was found to exist. When otherwise stated the growth rate in different regions varies in an orderly way, so that increase in green weight plotted against position yields a sigmoidal curve. For best growth of oriental types of tobacco, experience has shown that growth rates should be slow and uniform from the time the plants become established after transplanting until the end of harvesting. Under ideal conditions, therefore, for this crop, the characteristic growth

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Explanation of figures 5, 6

FIG. 5. Comparison of distributional growth curves of Izmir tobacco. One group of plants was topped and primed and the other was allowed to grow unmolested. Weight of internode and leaf combined is plotted against position. The weight increases progressively to a point of inflection at the median region and then it decreases progressively. FIG. 6. Comparison of distributional growth curves of leaves only of Izmir tobacco, otherwise as in figure 5. Vegetativeness is increased in response to topping and priming.

curve should approximate a straight line. At least two factors are involved in securing the degree of uniformity in growth rate shown herein. They are (a) the use of organic fertilizer instead of inorganic materials for the purpose of making nutrients slowly available, and (b) the selection for use as seed plants of those individuals that flower and fruit late, thereby tending to promote a long growth period. At present it seems doubtful whether it is practical further to modify the growth rate and thus to improve the quality of oriental tobacco or further to straighten the curves of growth shown in figures 2 and 3.

It is well known that the increments by which weight increases during growth are related to the weight of the organic mass producing these increments. Various investigators, recognizing the existence of this ratio "increment/mass" $\frac{(\Delta m)}{m}$ have attempted to express mathematically increase in assimilatory power or "net assimilation rate" (Williams 1946). For instance, Blackman (1919) likened such continual augmentation to money increasing at compound interest. He employed the equation $W_1 = W_0 e^{rt}$, where W_1 = final weight, W_0 = initial weight, t = time, and r, used exponentially, the rate at which material present produces new substance. Hence e^{rt} becomes the efficiency index. The similarity between growth and compound interest does not hold throughout the entire developmental period, however, as indicated by Glaser (1938), for the reason that, as time goes on, percentage production declines and eventually stops.

Other investigators, including Bernard, Chodat, Loeb, Robertson,[3] Hatai (1911) and Reed (1921) have considered rate of growth analogous to autocatalytic reaction, and in certain instances (Reed & Holland 1919) actual curves of growth have been compared with calculated (autocatalytic) curves. They assumed that growth rate is analogous to the velocity of a monomolecular autocatalytic reaction; or $V = \frac{dx}{dt} = k(a - x)$, where V = velocity of reaction, x = size of the organism at time t, and a = final size. But as x increases, the new substance produced also exercises a catalytic effect, hence $\frac{dx}{dt} = k'x$ $\times (a - x)$. By integrating, Pearl and Reed (1923) derived $\frac{1}{at} \log \frac{ax}{a-x} = k'$. When the organism is half grown ($a/2$), and hence when the reaction is half completed (duration being considered t'), by integrating, $\log \frac{x}{a-x}$ $= K(t - t')$. If this formula is applied to epochs in organic growth, the observed and calculated growth curves exhibit a high degree of "closeness of fit."[4] Such mathematical treatment appears, however, to have a limited use-

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[3] See Thompson, l.c., pp. 256–258.

fulness when applied to total growth and, as Thompson (1945, p. 261) has emphasized, the *modus operandi* of growth still remains unexplained.

As shown in figure 2, the green weight of substance produced increases progressively from the base of the stalk to the median stalk region, and then there is a progressive decrease. A similar relation between length of internode and position exists, as might be expected, although it is not shown, and if length is plotted against position the result is a sigmoidal curve.

To express growth in the manner employed in figure 2, in preference to plotting elapsed time against either green weight, dry weight, or height, does not conform with the usual procedure. No single method of expressing growth seems entirely adequate and all are open to criticism. One reason for this situation is that the moisture content of leaves, and presumably of internodes also, is known to differ at different stalk positions, the uppermost leaves containing the larger proportion of dry substance (Wolf & Jones 1944). Williams (1946), who studied the growth of *Phalaris tuberosa* L., concluded that during early vegetative development leaf area and leaf weight are satisfactory indices of growth but, all in all, amount of elaborated leaf protein is the more adequate index.

As regards responses induced by topping, Avery (1934) demonstrated that increased leaf size in topped tobacco plants was not due primarily to production of new cells but to increase in volume of cells already present. Petrie, Watson and Ward (1939) concluded that when plants are topped, shifts in leaf area and in dry weight, that tend toward counter-balance, are induced. They expressed the opinion that assimilates destined to produce the inflorescence in non-topped tobacco plants either remained in the leaves of topped plants or a portion of the food was translocated to the roots. Increased leaf size, induced by topping, was explained by Wolf and Jones (1944), as a compensatory response, tending to follow Le Chatélier's Law.

The present observations on rate of leaf expansion and on the duration of growth are opposed to generally accepted beliefs as expressed by Wolf and Jones (1944) and they do not confirm the findings of Berthold (1929)[4] with tobacco. The lack of accord on these matters may most reasonably be ascribed to the fact that leaves are not ready to harvest as soon as they attain mature size. But there remains the probability that differences in cultural practices with different types to tobacco may account for differences in rate and duration of leaf expansion.

Thompson (1945, p. 281) states that ''we are at present little able to explain the characteristic form of the growth curve but for it we may hope

[4] Berthold, T., l.c., p. 55. ''Die Wachstumsdauer der unteren Blätter der Pflanze ist am kürzesten. Mit zunehmender Insertionshöhe bis zu den mittleren Blättern verlängert sie sich. Zwischen den oberen und mittleren Blättern ist der Unterschied in der Länge des Wachstums meistens klein.''

to have some day a simple explanation." The fact, as shown in figures 5 and 6, that a shift in the curve, consequently a change in slope, can be induced by the removal of the floral-axis (topping) appears worthy of consideration in connection with this conclusion. The present observations leave no doubt that the change from an increasing to a decreasing velocity of growth of the tobacco plant is initiated by and controlled by mechanisms that attend the development of the flowers and seed pods. The primary causes are presumably hormonal, are produced by the tissues of the floral-axis, and these hormonal substances tend to inhibit "vegetativeness." Such interpretation assumes that the factors which regulate vegetativeness also are hormonal. From these assumptions it follows that the point of inflection of the differential curves in figures 5 and 6 marks the period of occurrence in the plant's development of an hormonal balance between "vegetativeness" and "reproductiveness." A small degree of support for this interpretation arises from observations like those of Priestley and Pearsall (1922), who noted in studies of growth of roots of *Tradescantia zebrina* Hort. and tomato that decrease in velocity of growth is coordinated with the initiation of lateral meristems.

On the other hand, the flowering and fruiting of plants has been explained on the basis of a change in nutritive balance. It has long been known that the change from a vegetative phase or condition to a reproductive one is accompanied by a change in carbohydrate/nitrogen balance. Fisher (1916), for example, reported that vegetativeness is characterized by a carbohydrate content that is relatively low in proportion to the nitrogen content (i.e. there exists a low carbohydrate/nitrogen ratio), whereas the ratio is high when plants are in the reproductive condition. Corroborative evidence of such change in equilibrium has been found to occur in tomato (Kraus & Kraybill 1918) and in wheat (Hicks 1928). There are some who interpret such change in carbohydrate/nitrogen ratio as the proximate cause of flowering, whereas others regard it as a more distally related factor in a concatenation of causes and effects. The interpretation of the latter group is strengthened by the fact that the carbohydrate/nitrogen ratio may not be uniform throughout the entire plant (Hicks 1938, Darkis et al. 1936). Quite commonly the samples used for analysis in appraising carbohydrate/nitrogen ratio have consisted of an aliquot from a composite of the entire plant rather than of particular parts or organs. When the samples are thus prepared the fact that chemical differences exist between organs or that such differences may be localized within an organ are quite completely obscured. This situation is shown in a striking manner by Andreadis et al. (1939) in their findings on the distribution of nicotine in the leaf of tobacco. They found that nicotine increases progressively from the base to the tip of the leaf, from the midrib to the leaf margin, and from the secondary veins toward the parenchyma tissues

most distant from these veins. These differences were of the order of twice to three times as much in one locus as in another.

In the light of the varied approaches that have been made to problems of growth of cells, tissues, organs, or of the ontogenetic development of any organisms in its entirety, and to the interpretations of such studies, it becomes axiomatic that growth is not to be regarded as a simple process, but as a complex of interdependent processes. There is little likelihood, therefore, that by means of a simple explanation, as some investigators believe, growth curves will come to be adequately understood. Rather it should become increasingly apparent upon reflection that, at least for the present, botanists should devote themselves to fundamental considerations of growth whether of roots, stems, leaves, flowers, or fruits, for by so doing they will contribute to a better knowledge of these growth complexes, and, as a consequence, it may become possible eventually to synthesize a broad, integrated growth principle or set of principles.

The change in absolute velocity of growth near the median stalk region may also be basic to the primary grading or classification of cured tobaccos into the two grades "lug" and "leaf." Lug grades of flue-cured tobacco are produced along the lower half of the stalk and leaf grades along the upper half, with some degree of overlapping in position occasioned by seasonal differences. The fundamental differences between these two grades are not believed, however, to be causally determined by environment but by internal factors. Leaves belonging to the lug grade arise under the dominance of internal factors governing vegetativeness and those to the leaf grades under the dominance of internal factors governing reproductiveness. These two groups of factors are not additive or augmentative in conditioning rate of growth but instead have a subtractive or oppositional effect.

The designations "lug" and "leaf" have been established for a long time among tobacconists. How and why they were introduced is controversial, and no real purpose is served at this time by statement or opinions pertaining to this subject. The fact remains, however, that there are chemical differences in composition between leaves of these two grades, and between leaves of their subdivisions, in the case of flue-cured tobacco, as shown by Darkis et al. (1936). These chemical differences, to be sure, reflect metabolic or physiologic differences during growth and maturation.

The straight portion of the curve, as regards oriental tobacco, is clearly all-important. Further increase in the production of quality grades of oriental types of tobacco would seem to be related to modification of cultural practices in such manner that rate of growth could be expressed as a curve that approximates through its entire length a straight line. To do this vegetativeness should be enhanced at the expense of reproductiveness. If this procedural approach is the correct one, then the "lug" zone might be further

extended, as should result, if flowering were delayed until essentially all the leaves had been primed.

If the critical point of inflection in the growth curve of tobacco is related to vegetativeness as opposed to reproductiveness, then for the first time a rather simple explanation is at hand for the characteristic growth curve of this species, and also for the first time there is established a morphologic basis for the primary grading of cured tobaccos.

These observations on growth of oriental types of tobacco also suggest that growth curves might become useful aids in determining the extent to which quality and yield of tobacco of any type may be modified by different topping practices, by spacing, by the application of fertilizers, or by other cultural practices.

SUMMARY

A study has been made of the rate of growth in oriental types of tobacco. As anticipated, mass plotted against time is found to yield a characteristic sigmoidal growth curve, but mass plotted against stalk position yields a distribution curve with a maximum in the median stalk region. The rate of growth of the stems or of expansion of the leaves when plotted against time also becomes a sigmoidal curve.

The duration of the period of expansion of leaves borne at different stalk positions is quite alike. Rates of leaf expansion, irrespective of leaf position, when based on final size, are also quite alike.

Removal of the floral-axis (topping) induces a change in slope of the growth curves as a result of disturbance of balance between the hormonal complex that controls vegetativeness and the complex that controls repro ductiveness.

The inflection of the growth curve at the median stalk position is interpreted as being causally related to opposition between the hormonal factors that control vegetativeness and those that control reproductiveness.

On the basis of such interpretation the leaves produced under the dominance of vegetativeness are in the "lug" grades, and under the dominance of reproductiveness, in the "leaf" grades. The proportion of lug and leaf grades and their quality are therefore, to a degree, controllable by cultural practices.

It is suggested that growth curves might serve to aid in the interpretation of results of various agronomic practices with tobacco.

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A PHYSICAL ANALYSIS OF GROWTH AND STRUCTURE OF SOME GRASSES

Otto Stuhlman, Jr.

An expanding phenomenon in time is usually associated with organic growth. As a first step there is the formation of new protoplasm, which is always accompanied or immediately followed by a permanent increase in the amount of non-living materials. Hence the growth of a plant consists primarily in the formation of new cells, of their enlargement and specialization, so that "a quantity of growth" implies more than a simple progressive increase in size with an accompanying increase in mass, or decrease in density. Accompanying the physical changes in form and structure must be changes in internal stresses, which must manifest themselves as variations in the elastic constants of the material as the structure develops.

In the growth process each section of the plant, in its development, has its formative stage, its elongative stage or "grand period of growth," and its stage of maturation. These dynamic aspects of the growth processes have been analyzed in great detail and with Buchanan (1) we may divide the whole period of growth into (a) a lag phase, (b) the phase of logarithmic increase, (c) the phase of retardation, (d) the stationary phase plus the alternate terminal phase of restoration (e), or decline (f).

These phenomena constitute the generalized interpretation of a typical sigmoid growth curve usually expressed in terms of length or mass units as the dependent variable (green weight, dry weight, or height) plotted on the y-axis, and time as the independent variable plotted on the x-axis. In general, if the total increment of growth is plotted against time, the resulting curve will be sigmoid in shape. On graphically differentiating such a sigmoid-shaped curve and then plotting the differential values as a function of time one obtains an asymmetric distribution curve, showing the increase in height, green weight, or size of leaves along the stalk of a plant, in terms of increments of time. The ascending phase of this distribution curve will be steeper than its descending phase. If, however, the sigmoid-shaped curve is symmetrical (S-shaped) so that the point of inflection, where the change from an increasing to a decreasing velocity of growth is indicated, lies halfway up the curve, then the distribution curve is also symmetrical and approaches the form of the bell-shaped probability curve.

Conversely, if a distribution of length, mass, volume or any other physical characteristic of a completed structure, in the form of a mature plant, is graphically represented in terms of any independent variable, it should

be possible to obtain from such data a growth-characteristic of that plant. Graphically integrating such a distribution curve will reduce it to a sigmoid-shaped growth curve, if the distribution curve is asymmetric, or to the S-shaped counterpart if the distribution curve is symmetrical.

The object of the analysis of grasses that follows is to show that the static physical or structural characteristics of a mature plant, after it has attained its "stationary phase," can be used to obtain certain physical growth characteristics, providing the physical measurements can be represented as a distribution curve, when the distribution is independent of the time element usually associated with a "quantity of growth."

Analysis of Arundo donax.   One of the most stately of the ornamental grasses found in the southern United States is the giant reed (*Arundo donax*), often reaching a height of 20 feet or more, and topped with a gorgeous green-gray tassel. This giant reed is a member of the *Class Monocotyledones*. The most prominent physical feature of its slender stalk is its segmental division into a large number of tubular joints (internodes) between stiff circular nodes. During growth the elongation of the internodes takes place at their bases where they contact the nodes and the base of the sheaths which surround the cylindrical internodes.

On examining a very tall mature reed, one is struck by the architectural effects of the distribution of its nodes along the stem. The internodes are very short at the base, increase in length as we ascend the stem, and reach a maximum length at a point about one fourth of the way up the structure. This characteristic increase in length is followed by a distribution in which the internodes are progressively shorter until they terminate in an enormously elongated floral-axis surmounted by the tassel.

In this ascending distribution of change in the physical characteristics of the internodes we have a static, but complete picture, of a phase of "exponential increase," followed by the static structure reflecting a phase of retardation.

Figure 1 shows typical variations in the length of the internodes of *Arundo donax* in terms of the ascending position of the internodes along the stem. The data represent arbitrary samples taken from the same patch of grasses during each of three consecutive years. We immediately note that the lengths of the internodes diverge more rapidly in the lower segment (phase of exponential increase) than they converge in the upper segment (phase of retardation). The discontinuity between the sequences is localized at the maximum of this distribution-in-length curve, which is usually found to be associated with the center of gravity of the reed, and will be referred to as the *critical point* or inflection point of the stem.

If we determine the dry or green weight of the internodes with or with-

out attached leaves, and then graphically represent the green weight of the internodes and leaves, in terms of their ascending order, we obtain similar asymmetrical distribution curves, as shown in figure 2. They all indicate that the maximum length, mass, or volume (fig. 3), for any given stem, is always a common attribute of the same node lying at the *critical point* or point of inflection on the sigmoid growth curve.

If we plot the total increment of length, of the same stem, as a function of the position of the internodes in ascending order, we obtain a typical sigmoid curve, as shown in figure 4, whose point of inflection coincides with the maximum (critical point) of the distribution curve of figure 1. The same type of sigmoid curve is obtained if a distribution curve of the form shown in figure 3 is graphically integrated, as shown by the broken curve in the same figure. Figure 3 illustrates the changes in volume of the hollow internodes of *Arundo donax* in terms of the positional number of the internodes in ascending order, up the stem.

If the form of the sigmoid curve, showing the variation of some physical factor as a function of time, is accepted as representing the dynamic growth characteristic in time, it should follow that if an identical graph is obtained by integrating a curve representing a distribution in which the dependent variable (y-axis), is the same physical quantity as represented in the sigmoid curve, then the independent variable (x-axis) used in the distribution curve must be a function of time. In this way we may justify the use of the position of the internode, or distribution of substance along the stem as the independent variable, provided we limit the results to a qualitative interpretation.

Thus a curve depicting the mass, length, volume or any other physical characteristic distribution, in a completed static structure (mature plant), as a function of the position of the internodes, the graph should indicate the same growth characteristics as its comparable sigmoid growth curve (fig. 3).

Presentation of the data as a distribution curve has the advantage of showing more precisely where the growth changes from its major phase of exponential increase to its phase of retardation. Any irregularities occurring in either growth phase are also more easily identified in a distribution curve than in a sigmoid growth curve.

Note for instance what appear to be irregular departures in the normal phase of retardation of the total mass of internodes with attached green leaves shown in curve A of figure 2. The enlarged insertion shows how these apparent irregularities near the twenty-first internode are in reality a local change from the phase of retardation to a phase of exponential increase followed by an appreciable compensation of greater retardation in the immediately following group of higher internodes. Such sudden changes in slope were always found associated with the presence of a bud or branch. Apparently such changes in slope must be attributable to the development
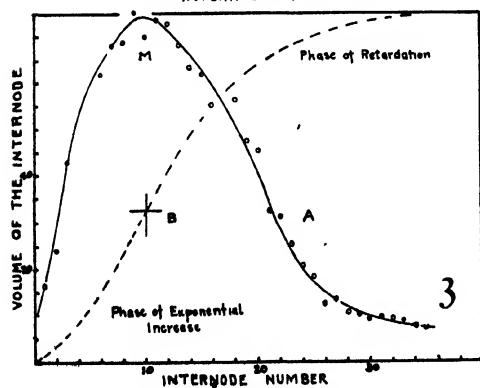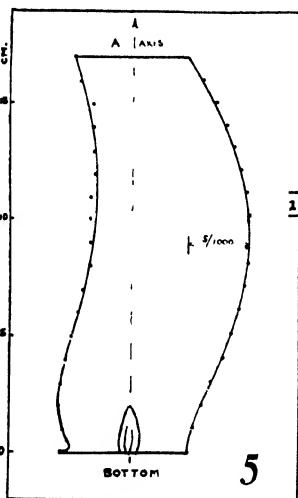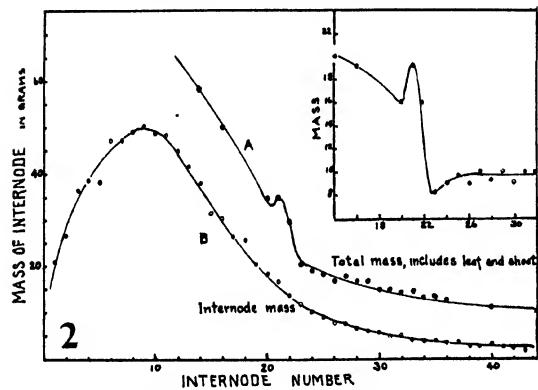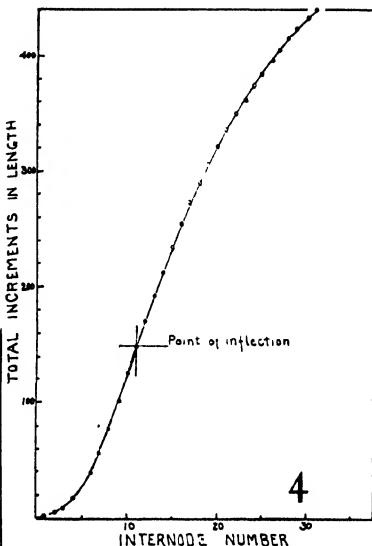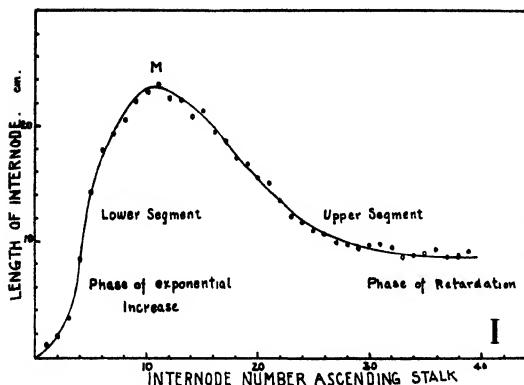
of a secondary structure on the main stalk. The secondary structure apparently uses part of the available energy for its own growth, thus changing the rate of growth in subsequent higher internodes and their attached leaves.

In support of this view may be cited the critical analysis of "the compound interest law and plant growth" by Blackman (2), who pointed out that the reproductive development usually occurred during the period of deceleration in rate of increase in dry weight. The observations of Priestley and Pearsall (3), indicated that the decrease in the velocity of growth of the roots of *Tradescantia zebrina* Hort. and tomato could be coordinated with the initiation of lateral meristems. Pearsall (4) in his studies of the growth of cotton also attributed the decreased rate of growth of the stem to flowering.

If the development of a bud or branch can cause local changes in the slope of the growth curve, it should follow that the predominant change in slope identified with the *critical point* located by the maxima of the distribution curves, should be attributed to similar but more fundamental internal changes in the plant structure.

Euler's Column Theory Applied to Internodes. If the foregoing hypothesis is biologically acceptable it should follow that radical changes in the internal composition and structure of the stem, as reflected by changes in the elastic modulus of the material, should be found associated with those internodes which lie at the maxima of the length, volume, and mass distribution curves.

To explore the possible changes in internal structure of the nodes along the stalk two structural tests were applied involving the modulus of rigidity of the material. The first involved examining the internode as a unit column, placing it under a compression load until fracture took place. The second,

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Explanation of figures 1–5

FIG. 1. A distribution curve showing typical variations in lengths of the internodes of *Arundo donax* in terms of the ascending position of the internodes. *M*, the maximum, is the point of inflection of the equivalent sigmoid growth curve. FIG. 2. A distribution curve showing typical variations in mass of the internodes of *Arundo donax*. A, shows variations in total green weight, which includes weight of internodes, leaves and any attached buds. B, shows mass of internodes after leaves and buds have been removed. Insert shows in detail the change in slope of graph A, near the twenty-first internode due to the presence of a bud. FIG. 3. A, a distribution of volume of the internode as a function of internode position. B, the graphical integration of curve A produces a typical sigmoid growth curve. Point of inflection B coincides with the maximum of the distribution curve. FIG. 4. A typical sigmoid growth curve obtained by plotting the total increments in length. The measurements were obtained from the same plant that is represented in figure 1 as a distribution-in-length curve. FIG. 5. Contour of the *x*-axis and *y*-axis of a 17 cm. internode of *Arundo donax*. The vertical scale shows that measurements were made at centimeter intervals. The horizontal axis is on a scale 1000 times greater than the vertical centimeter scale. The contours have the form taken by an elastic column under compression.

a more rigid and quantitatively interpretable process, involved a determination of the elastic modulus of compression and then the evaluation of the compression modulus in terms of the "slenderness ratio" of the columns. This will give critical internal structural information about the stiffness of the internodes in terms of ratio of length to radius of gyration. From such data we can then draw quantitative conclusions about changes in internal structural properties of the internodes without resorting to the procedure of plotting a distribution curve in terms of the position of the internodes.

It is proposed to examine the internodes under compression and determine whether their yield stresses and their fracture stresses radically change as we pass from the lower segment through the *critical point* to the upper segment of the stem.

The internode-structure closely approaches the form of cylindrical shells. Such a structure when put under compression in the direction of the longitudinal axis will at first bend and then fracture. This type of failure is that investigated by Euler in 1757, who showed that the strength depends on the ratio of length to radius of gyration of the cylindrical shell, and on the modulus of elasticity of the material.

Euler sought, on theoretical grounds, to determine the least value that an axial load P (pounds or kilograms) must attain so that an elastic slender column will start to bend. Since a column buckles when the load is increased only a small amount above this value, then P is also the minimum load that the column can resist, so that the axial compression will make the column fail by buckling and not by crushing. This critical load for an ideal pivot-ended column was shown by Euler to be equal to

$$P = \frac{\pi^2 E I}{l^2}$$

where P is the axial load in pounds, E the elastic modulus of the material expressed in pounds per square inch, I the moment of inertia of the cross-section of the column about the neutral axis, and l its length. If a is taken as the area of the cross-section and r the radius of gyration of the cross-section about the neutral axis, then Euler's column formula may be written

$$\frac{P}{a} = c \frac{\pi^2 E}{\left(\dfrac{l}{r}\right)^2}$$

where l/r is called the slenderness ratio of the column, and c is a constant depending on the end conditions of the cylinder when the pressures are applied.

From Euler's equation we may expect P/a to be inversely proportional to the square of the slenderness ratio, $(l/r)^2$. By an increase in slenderness can be meant (a) an increase in l with r constant, (b) a decrease in r with l constant, (c) or an increase in l and a decrease in r.

' These theoretical results show that the only internal property of the material on which P depends is E, the modulus of elasticity, or stiffness (not rigidity) of the material. Since the idealized end-conditions cannot be reproduced with plant structures as successfully as in the supports of columns used in engineering practice, and since the complexity of the fiber-structure of the shaft of the reed must certainly be taken into consideration, one can expect only limited agreement between an ideal cylindrical column and actual cylindrical plant structures.

The first object is to determine to what extent the external structure of the internodes of *Arundo donax* resemble the idealized mechanical configuration of a uniform cylindrical column with proper end supports.

If an internode is to be used as an ideal test-column then the external and internal radii must be constant throughout its length. The external contour of the internodes were therefore explored by means of a sensitive spring gauge, which was calibrated to read 1 1000 inch. The internal radius was explored by measuring the thickness with a screw gauge. To determine the degree of departure from a round column the internodes were mounted on a plane steel surface, and the change in height along a geometrical longitudinal axis was measured at centimeter intervals. The internode was then rotated to a 90°, 180°, and 270° position and variations in contour were also determined along these longitudinal axes. For convenience of notation the *A-axis* is taken as the longitudinal line on which the bud is located. The *y-axis* lies 90° anticlockwise, followed by the *B-axis* and *x-axis,* at 90° intervals. Figure 5 shows a typical series of contour measurements along the x and *y-axes* of an internode. This form of contour is typical of most of the longer internodes. The contours are typical harmonic curves, similar to those developed by elastic metal columns subjected to compressional forces. The internode, during its growth, seems to have been subjected to longitudinal stresses which in effect act as a deterrent to prevent the column from being perfectly cylindrical, or the leaf at the node seems to have acted as a circular constrictor to produce these mathematically correct distortions.

The over-all shape is very nearly cylindrical, as one can see from figure 5 where the length is plotted in terms of centimeter intervals and the change in contour in 1 1000 inch units. The maximum over-all departure of this 17 cm. long internode, from a true cylindrical column, is a bulge of 5/1000 inch located near its midpoint.

Figures 6, 7, 8, and 9 show how thirty-eight successive internode-contours progressively change as we ascend the stem. The long internodes of *Arundo donax* lying above and below the *critical point,* that were subsequently examined, never departed more than 6/1000 inch from true cylinders, a variation of not more than ¼% from a true circular column.

To a fair degree of approximation we can therefore proceed with the
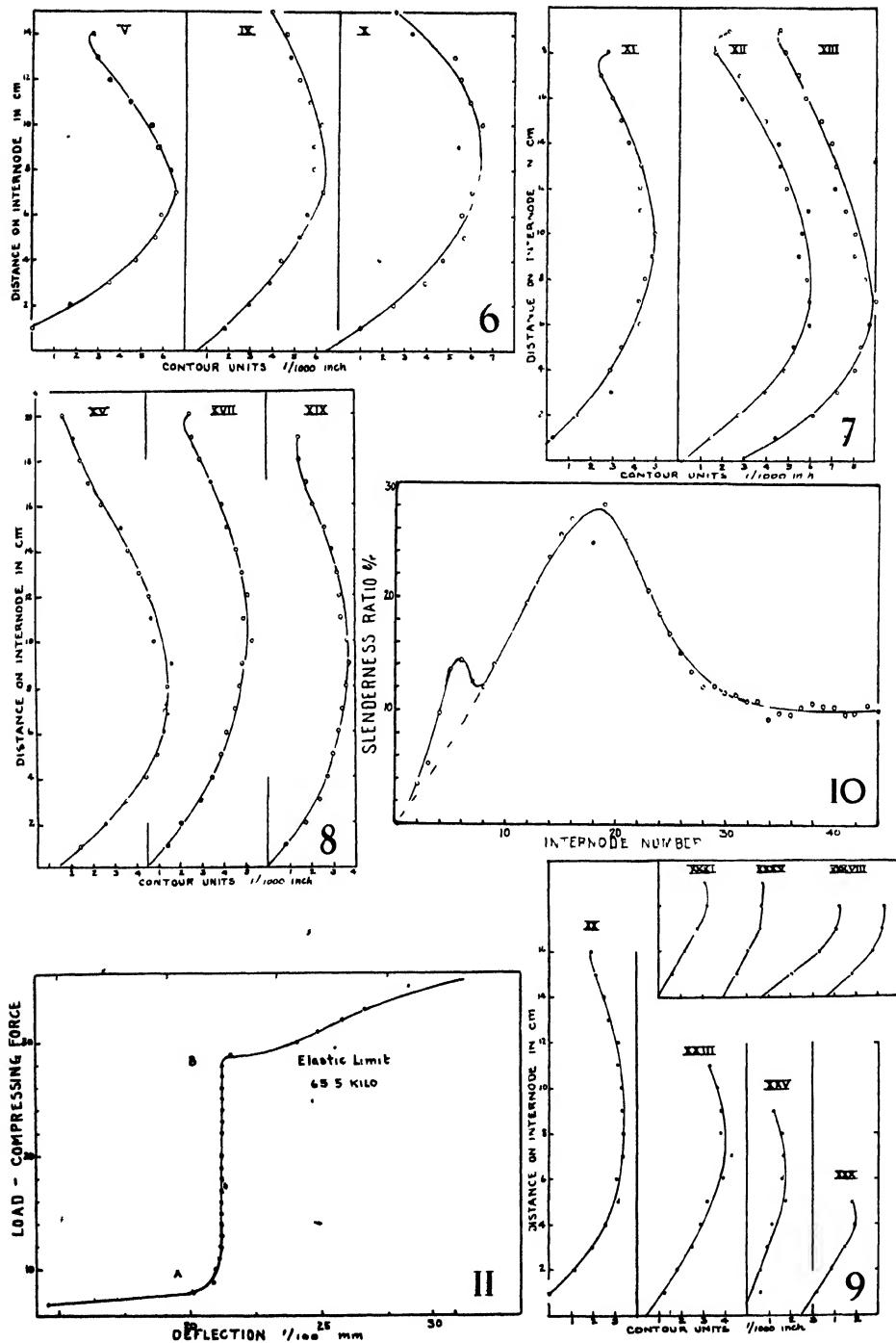
compression tests assuming that the middle half section of an internode may be treated as if it were a hollow cylinder conforming nearly to Euler's ideal cylindrical contour.

Fracture Tests.   Values of the slenderness ratio, l/r, were next measured and identified with the internode position along the stalk. Here l represents the distance between two consecutive nodes and r the radius of gyration, i.e. $\left(\dfrac{(R_i)^2 + (R_c)^2}{2}\right)^{\frac{1}{2}}$ where R_i and R_c are the internal and external radii respectively. Typical results are shown in figure 10. The lower segment shows that the slenderness ratio increases as we ascend the stem, attains a maximum at the same *critical point* as the other physical factors and then decreases less rapidly in magnitude toward the top. This verifies the assumption that the stem, considered as a unit structure, is also characteristically divisible into two segments having very different internal structural characteristics.

In order to explore this possible internal structural difference, the stems were cut into cylinders with a node at each end. Under these circumstances only every other internode could be examined. By this method nearly similar nodal end supports to all the columns were provided. Such columns were examined for ultimate strength (breaking load per original cross-section area) with a testing machine whose compressional force could be increased in 2-pound steps.

Ultimate strength is defined as the greatest unit stress that the column can stand in compression without breaking. This is designated as P/a where P is the load in pounds and a the cross-sectional area of the hollow cylinder in square inches.

The results were unsatisfactory because of the limited amount of data available from any one stalk, since alternate internodes had to be discarded. Much of the data was also found to be unreliable because of the great scatter of the points, but general trends were thus established. Later it was noted that the presence of lateral buds introduced large experimental errors. Another difficulty arose for the reason that the measurements could not be

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Explanation of figures 6–11

FIGS. 6, 7, 8, 9. These graphs show the progressive change in shape of the right hand vertical contour (fig. 5) of seventeen of the thirty-eight internodes of a tall stem of *Arundo donax*. Note that the contours of the enumerated internodes bulge less as we ascend the stem. They simulate the physical appearances of a cylindrical rod under pressure, responding to a progressive decrease in vertical load. FIG. 10. The slenderness ratio (l/r) of length to radius of gyration of the hollow cylindrical section of the internode is shown to have the form of a typical distribution curve. The departure from the smooth curve near the origin is due to the influence of a large bud. FIG. 11. A typical graph showing the progress of the flexure of a longitudinal axis of an internode of *Arundo donax* under compressional test. B is the yield point. In this sample, fracture occurred when a load of 65.5 kilograms was applied.

repeated, due to the destruction of the material on fracture. In general, however, the trend of the data indicated that the lower segment always showed a decrease in ultimate strength with increase in slenderness ratio while the upper segment showed a decrease in ultimate strength as the slenderness ratio decreased. The stem must therefore be rather stiff at its base, become less stiff as we ascend through the middle third, where it passes through a critical internal structural phase. Here the internode suddenly becomes even more stiff than those at the base and then they progressively become less stiff as we near the upper internodes.

These results again support the assumption that the *critical point* of the distribution curve indicates a unique structural change due to some internal economy of the plant.

From Euler's equation we would expect the fracture tests of the internodes to show that the product $(P/a)(l/r)^2$ is constant, assuming E, the modulus of rigidity, to have the same value for all internodes. The available data showed however that this relation was not constant for all the internodes in any given plant. Under the circumstances it became necessary to determine the modulus of rigidity for every internode as we ascend the stalk to verify the suspicion that the trend in the value of E must change radically at that internode identified with the apex of the distribution curve. This should lead to quantitatively interpretable results from which the internal structural quality of the plant material can be evaluated.

Tests for Modulus of Elasticity.   If we start with a simple short solid cylinder and subject it to an end-load having a very slight eccentricity, the short cylinder may be assumed to be in pure compression since its bending action may be neglected. If the cylinder is thought of as becoming longer (more slender) the tendency to bend becomes greater, until in the limiting case the column is so slender that bending or flexure is the only action that need be considered. The contour of the elastic curve that is generated as a result of the compressing force is similar to the shape taken by a slender stick when it is vertically compressed between the palm of one's hand and the ground which supports its lower end. Any slender internode of the reed must bend in a similar manner if subjected to sufficient pressure directed vertically downward.

To determine the magnitude of the modulus of digidity it is necessary to measure the buckling that takes place at right angles to the longitudinal axis of a cylindrical sample cut from the middle section of each succeeding internode. If these hollow cylinders are uniformly compressed in a testing machine, in the axial direction, symmetrical buckling with respect to the axis of the cylinder may start at a certain value of the compressive force which is called the critical load.

A sensitive (1/1000 inch or 1/100 mm.) spring gauge was used to measure the buckled deflection. The graph showing the relation between stress (load in pounds), and the strain (deflection in multiples of 1/100 mm. or 1/1000 inch), is shown in figures 11 and 12. The portion AB is a straight line, but BC is slightly curved, the lower side being the concave side. The point B, at which the strain begins to increase greatly for very little increase of stress, is called the yield point.

Within the portion AB of the strain diagram the stress is proportional to the deformation produced, and the material may be considered perfectly elastic. The fact that the experimental values between A and B can, to a close approximation, be considered as falling on a straight line, shows that within the elastic limit the deformation of the cylinder is proportional to the stress producing it, thus obeying Hooke's law. This can be restated by saying that the ratio of the unit stress to the unit deformation is a constant, and this constant is known as the modulus of elasticity E, expressed in lb/in.^{2} or kgm/mm.^{2} Geometrically E is the slope of the line AB in figure 12.

Figure 13 shows the values of E thus obtained from the successive internodes of a single plant (*Arundo donax*) in terms of the height above ground at which the sampling took place.

The modulus of elasticity of the lower internodes was found to be about 1,600,000 lb/in.^{2}, dropping to 400,000 lb/in.^{2} for internodes situated higher up the stalk These values are of the same order of magnitude as those reported in the U. S. Forestry Circular No. 15 for 32 samples of wood (40% moisture) varying from 2,500,000 for hickory to 910,000 lb/in.^{2} for white cedar. Schwendener (5) reports that the elastic modulus of bast from eight selected monocotylons is on the average about 2,000,000 lb/in.^{2}

Since each successive internode furnished a test-column from which E. was determined, the values of E could therefore be identified with the position of the internodes in addition to their linear height above the ground. This made it possible to show the correlation between the lengths and position of the internodes and their changing values of E. These relations are shown in figure 13, which also shows that the maximum of the distribution-in-length graph coincides with the minimum value of E of the elasticity curve.

The measurements indicate that the first internode possesses the greatest modulus of elasticity. As we ascend the stem the modulus of the internodes decreases as the internodes increase in length. The magnitude of the modulus passes through a minimum where the increase in length changes to a regression in length of the internodes. This minimum value of E can always be correlated with the *critical points* of the distribution curves.

Of special significance is the secondary decrease in the value of E, which was always found when a bud appeared at a node. This is illustrated by the
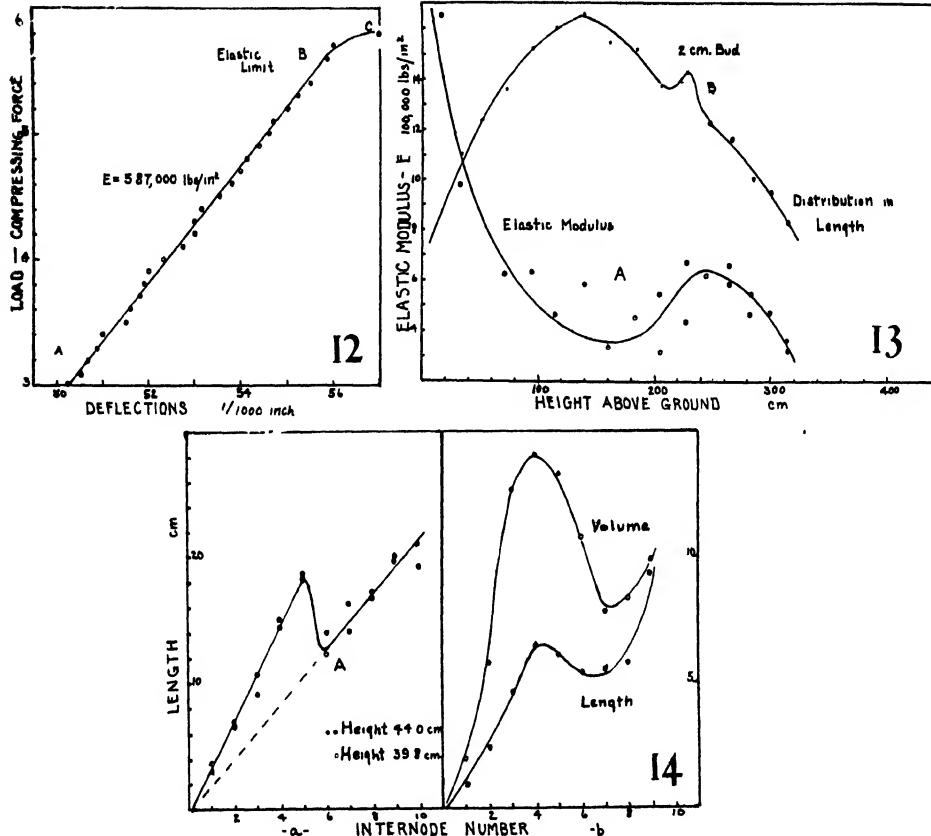
FIG. 12. An example of the flexure produced in a cylindrical test sample taken from the middle section of an internode of *Arundo donax*. Note how closely the data (*AB*) follow the straight line representing Hooke's law. These are not typical data but an illustration of the best results that were obtained from samples cut from the internodes of *Arundo donax*. Beyond the elastic limit *B*, the departure from Hooke's law sets in as shown by *BC*. FIG. 13. Curve A shows the general trend of the values of the elastic modulus *E*. Note that the minimum value of *E* coincides with the maximum value of the length-distribution curve for the same stem. The presence of the 2 cm. bud at *B* is reflected in the elastic modulus curve by the bulge at the sixteenth node. FIG. 14. a. Two nearly equally tall samples of Johnson-grass show identical distribution in-length curves. The ascending phase of the deceleration in the upper segment at *A* is correlated with the appearance of branches at the sixth and subsequent higher nodes. b. The distribution in-length and volume curves show identical forms despite the resurgence of growth at the seventh internode.

sudden decrease in E at the sixteenth node, with which a 2 cm. bud was associated. This latter decrease in the value of E shows that the presence of a secondary structure, a lateral bud, reduces the stiffness of subsequent internodes because of modifications in internal physical characteristics, probably brought about by a shunting of some of the food supply into the attached bud.

If the presence of a bud can be correlated with a definite change in the structure, as reflected in the change of the modulus of elasticity, it follows that a much greater change in this elastic constant must indicate a more profound internal change in the structure of the main stem. Such a profound change must therefore be associated with the critical change in the lengths of the internodes, correlated with the minimum of the elastic modulus graph.

From a physical point of view this critical change, at the twelfth node (fig. 13), from a decrease in stiffness to an increase in stiffness must therefore indicate the origin of a change in the internal structure. Since no external structure, aside from the leaf growth, is ever found at this critical position, one might attribute the increase in the value of E to the inception of the floral axis at a node located within this critical region. The subsequent coaxial growth of the stem would then reinforce the structure while draining the food supply to inhibit the growth of the internodes. This situation would account for the increase in the value of the modulus of rigidity (stiffness) of subsequent higher internodes, after which the stiffness factor would again be reduced due to the presence of external secondary growths along the stem.

Those samples which had buds associated with the higher nodes showed recurring decreasing fluctuations in the values of E.

Due to the high elasticity of the material it was often possible to make two consecutive measurements of E at two diametrically opposite places on the cylinder, so that occasionally two values of E could be obtained for the same column. In such instances (fig. 13) the graph was traced through the mean values of E in order to show the general trend of the elastic modulus.

Analysis of Sorghum halepense. In support of these analyses of *Arundo donax* we may examine the structure of Johnson-grass (*Sorghum halepense*).

The samples of Johnson-grass were gathered at random along a one-mile path during July and September. Figures 14a and 14b show the distribution in lengths and volume of samples posessing very few branches. Figure 14 indicates the distribution in length of two nearly equally high grasses picked from a random collection. The uniformity of these samples is shown by the average graph drawn through the mean values of the lengths of the internodes. Figure 14b shows the distribution in length of the internodes as com-

pared with their volumes. Their diameters were measured with a vernier caliper to the nearest tenth millimeter. The distribution is similar to that found for *Arundo donax*, in that the maxima of the length and volume distribution curves are identified with the same internode. The ascent of the accelerating phase is, however, more linear, and neither does the deceleration phase proceed to the top of the stem as in *Arundo donax*, but always passes through a minimum after which a secondary acceleration phase sets in, which terminates in the highest internode. This secondary accelerating growth phase has a smaller slope, i.e. its rate of growth is smaller than the primary growth phase of the lower segment. To a fair approximation both accelerating phases seem to have a common origin, which is located through extrapolation, at the ground level of the plant.

Analysis of Bamboo. That this resurgence in growth activity is not a unique phase confined to the higher internodes of the upper segments of Johnson-grass is illustrated in figure 15, graph A. This shows the distribution-in-length of the internodes in bamboo, as modified by the presence of branches. Here the resurgence takes place in the lower segment. It is correlated with the appearance of the first branches. Yater, it appears again at the node carrying two, and again higher up when the node has associated with it three branches. This is shown in greater detail in the insert of figure 15c where the graph shows how the length of the lower internodes, just emerging from the root, rapidly elongates. With the appearance of a single branch at the seventh internode the length drops to a lower value, indicating the beginning of a slower rate of growth. It seems to indicate that the external growth is dissipating some of the available energy and hence the rate of growth of the stem from this point upward is reduced, as indicated by the change in slope. This critical retardation is also present where the change-over from a one-branch to a two- or a three-branch growth appears at the nodes, as illustrated in graph B of figure 15. The over-all shape of the growth curve of the plant as a whole has, however, not been submerged. It still possesses, despite these superimposed secondary growth phenomena, the fundamental characteristics of the basic distribution curve.

The maximum of the distribution curve is hence indicative of a profound internal structural change, whose existence is not influenced by any secondary growths located either on the ascending accelerating phase, or descending retardation phase of the plant.

Analysis of Arundinaria macrosperma. Similar results are exhibited by the growth curves obtained from southern cane (*Arundinaria macrosperma*).

Without further comment figure 16 is presented to show how the lengths and volumes change with the internode spacing along the stem of this cane.
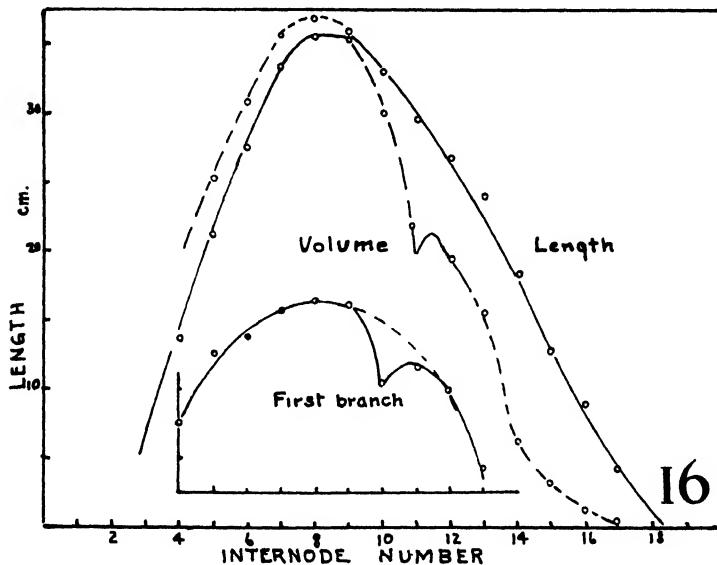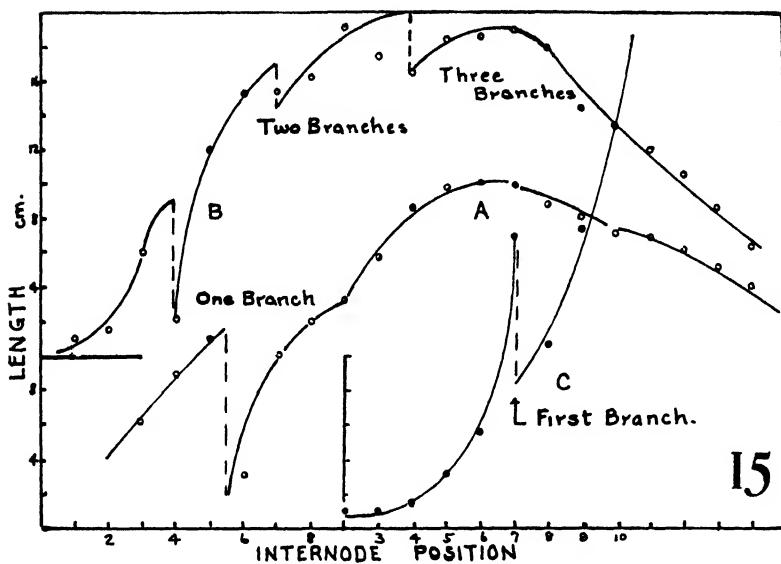
Fig. 15. Distribution-in-length curves for bamboo. A shows how the discontinuity in the ascending phase is correlated with the appearance of branches. Figure 10 shows a similar variation due to the presence of a bud along the stem of *Arundo donax*. B shows how the over-all contour of the distribution curve is maintained despite the variations due to the presence of one, two and eventually three branches per node at the higher node levels. C shows the detailed variation in length due to branching. These data were obtained from three different plants. Fig. 16. An example of the length and volume distribution curves of *Arundinaria macrosperma* with the local effects of branching. Note how the extrapolation of the volume or length curve indicates the maximum or self-limiting number of internodes that can be attained at the close of its growth season.

The first branch of this grass was usually associated with the descending phase of the distribution curve. The insert in figure 16 shows how the presence of the first branch effects the growth rate of the stem in its decelerating phase. The data also indicate no departure from the general contour of the distribution curve due to local changes in structure of the plant.

SUMMARY

The distribution in growth curves for the four grasses, *Arundo donax*, *Sorghum halepense, Arundinaria macrosperma* and a variety of bamboo are shown to indicate a higher degree of sensitivity for detecting changes in the rate of growth, than the conventional sigmoid form of the growth curve. The greater resolving power of the distribution curve allowed one to evaluate readily internal physical changes in the structure of these grasses in terms of the presence of external physical growth characteristics.

From the internal structural evidence, in the form of the modulus of rigidity of the successive internodes along the stem, it was shown that minor changes in value of the modulus could be correlated with the presence of branches at the nodes. The major inflection at the maximum of the distribution in mass, length, volume or slenderness ratio curve was correlated with the major change in the values of the modulus of elasticity. These latter changes were attributed to the inception of the floral axis at the node lying nearest to the maximum of the distribution curves. It was found that branching did not effect the over-all contour of the distribution curve. Instead, branching produced only local changes in the contour of the distribution curves. The appearance of a branch or bud at a node depressed the rate of growth and locally changed the elastic modulus. If the branch appeared at a low node the depression of the rate of growth was more marked than if the branches appeared at an upper node.

The data support the assumption that the completed static structure of a grass, when analyzed in terms of the physical attribute of the internodes as a function of the numerical position of these internodes along the stem, reflected the growth-in-time history of the plant.

Since none of the available data showed the accelerating phase of growth to be representable by a simple logarithmic function, it is suggested that the departure is due to the presence of secondary growths, which distort the expected ideal functional relation.

The modulus of elasticity of *Arundo donax* was found to be about a million pounds per square inch, comparable to that of white cedar. Southern industries are probably not aware of the economic value and strength of this material.

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LEAF ANATOMY OF STREPTOCHAETA AND THE RELATION OF THIS GENUS TO THE BAMBOOS[1]

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In connection with the study of the morphology of *Streptochaeta*, it was pointed out to me that the leaf-blades of *Streptochaeta spicata* contain a special kind of tissue composed of greatly enlarged cells which collapse at maturity. The study of these cells was approached with two objects in mind. In the first place the cells are of interest from the standpoint of development because of their extremely large size and the uniform way in which they collapse. In the second place the presence of similar tissues in bamboos and grasses of uncertain affinities presents an interesting problem in phylogeny. Brandis (1907) has already described the characteristic pattern of bamboo leaves and has shown that leaves of members of the Phareae are very similar. I have been able to add *Streptochaeta, Anomochloa, Pariana, Lithachne, Mniochloa,* and *Raddia* to the list. Not only are the enlarged cells present in the leaves of all these grasses, but their leaf anatomy is so strikingly similar that one is forced to consider the possibility of relationship among them.

Living material of *Streptochaeta spicata* Schrad. and *Pharus latifolius* L. was available from plants growing in the Indiana University greenhouse. Through the kindness of Mrs. Chase of the National Herbarium, pieces of leaves were obtained from herbarium specimens of various species of bamboo and of *Pariana zingiberina* Doell, *Leptaspis angustifolia* Summerheyes and C. E. Hubbard. Dr. Smith of the Gray Herbarium has generously provided herbarium material of *Mniochloa strephioides* (Griseb.) Chase, *Raddia brasiliensis* Bertol, *Lithachne pauciflora* (Swartz) Beauv., and several species of bamboo. Fixed material of *Olyra heliconia* Lindm., *Pharus parvifolius* Nash, and *Streptochaeta Sodiroana* Hack. was obtained from the collection of Dr. Weatherwax. Although scarcity of material prohibited my securing a piece of the only leaf of *Anomochloa marantoides* Brongn. in the National Herbarium, it was possible to discern the presence of the large mesophyll cells with the aid of a hand lens. Since most of this study dealt with leaves of *S. spicata,* the bulk of the discussion will be devoted to that species.

The appearance of *Streptochaeta* is very unlike that of grasses native to the temperate zone. Like many tropical grasses it bears leaves whose blades

are large and broad. In general *S. Sodiroana* is a much more robust plant than *S. spicata*. The culms of the former stand about 1 meter high and its leaves average 25 cm. × 7 cm. whereas *S. spicata*, whose leaves are 12.5 cm. × 4.5 cm. on the average, stands about 0.75 m. high. In both species there is a short, pubescent petiole between the leaf-blade and its sheath. Brandis (1907) pointed out that the midrib of bamboo leaves is usually larger than the other veins and contains several vascular bundles. The midrib of *Streptochaeta* leaf-blades is very distinct and in cross section protrudes from the upper side of the blade in the form of a keystone which becomes less noticeable towards the tip of the leaf. In *S. Sodiroana* numerous vascular bundles occupy the peripheral zone of the midrib, while two bundles, one dorsal to the other, lie in the center. The peripheral bundles are absent in *S. spicata*, although the two (sometimes three) median bundles are present. Although all grass leaves are characterized by parallel longitudinal vascular bundles, the veins in the leaf-blades of most of the generally recognized tribes are connected occasionally by cross veins. In the leaves of most bamboos, *Streptochaeta* and the few genera mentioned above, these cross anastomoses are characteristic.

Transverse sections of the leaf-blade (fig. 1 C) reveal a pattern which emphasizes its resemblance to leaves of bamboo (fig. 3 C). When the leaf is mature, it contains six layers of cells: the upper and lower epidermis, three layers of chlorenchyma, and a special kind of tissue composed of very large cells. The upper epidermis consists of flat, laterally lobed, elongated cells interrupted by occasional stomata and trichomes. Stomata in the lower epidermis are more numerous and are arranged in longitudinal rows on each side of the longitudinal veins immediately above the special large cells. Next to the lower epidermis there is one layer of green cells, and below the upper epidermis there are two layers. These mesophyll cells are irregularly lobed or cleft and are so arranged that they surround numerous intercellular spaces. The upper and lower regions of chlorenchyma are separated by the layer of enlarged cells. These cells are elongated transversely to approximately 4–6 times their height.

A cross section does not present a continuous row of enlarged cells across the leaf, but an alternation of these cells (fig. 1 C, *emc*) with lobed chlorenchyma cells (fig. 1 C, *cch*) and sheathed vascular bundles (fig. 1 C, *p*). There are usually four cells between the vascular bundles; two large cells separated by two chlorenchyma cells, although sometimes there may be only one or as many as three chlorenchyma cells or all the cells between the longitudinal veins may be enlarged. The bulliform cells occur just above the green cells which separate enlarged cells.

A longitudinal section of a leaf of *Streptochaeta* presents a very different picture (fig. 1 B). In *S. spicata* the side walls of the cells, which in cross
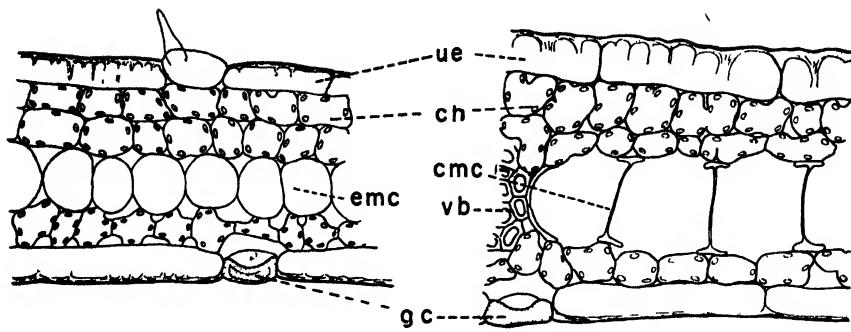
A

B
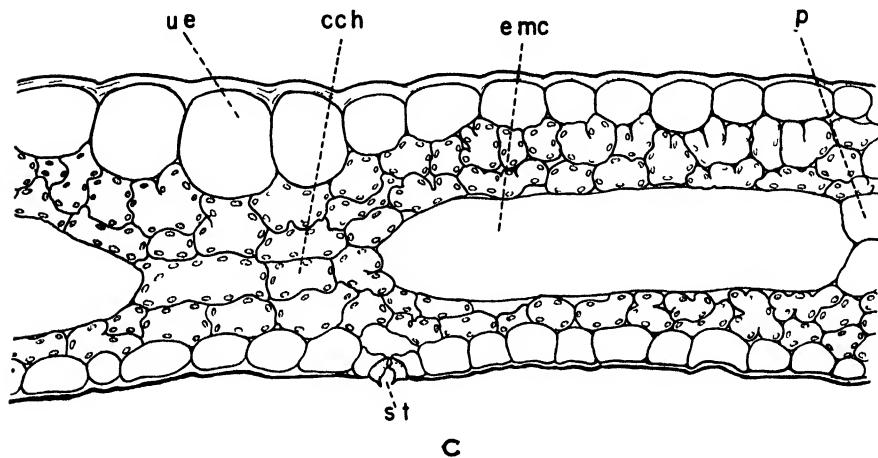
C
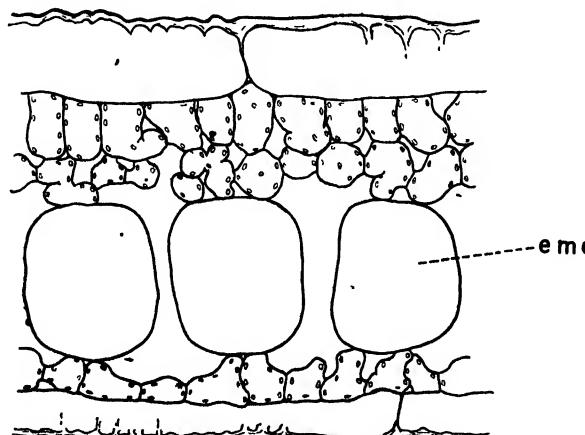
D

section are elongated, are collapsed in such a way that the tissue appears as a row of "I's." Between the collapsed cells there are large spaces which connect with the intercellular spaces of the chlorenchyma and thence to the stomata. The enlarged cells are devoid of chloroplasts and contain only dead cytoplasm. The large cells fail to collapse in *S. Sodiroana*. They die, but instead of caving in, they become very round and separated from one another (fig. 1 D), although they remain attached to the mesophyll above and below, and to the connecting chlorenchyma and the vascular bundles at either end. This same configuration is also found in the leaf-blades of *Pharus latifolius* except that the large cells do not become separated to the extent that they are in *S. Sodiroana*. Occasionally the cells will collapse, in which case they form the typical I-beam structure. In bamboos which have been examined the large cells collapse as they do in *S. spicata*.

Studies on the ontogeny of the leaf of *S. spicata* reveal that some of the cells which are destined to collapse are determined soon after the leaf-blade is formed and arise in a definite relation to the longitudinal veins. Early in development the blade is composed of five layers of actively dividing cells. Differentiation of the main longitudinal vascular bundles occurs first and the other bundles develop later. When the leaf blade is about half of a millimeter in width (fig. 2 A), only seven or nine bundles of provascular tissue can be discerned in cross section, whereas there are approximately forty bundles in the fully developed leaf. As the secondary veins develop from the middle layer, the cells adjacent to them begin to enlarge vertically at first and then laterally (fig. 2 B). The enlargement of these cells seems to keep pace with the rapid increase in number of cells in the surrounding tissues and consequent increase in dimensions of the growing leaf. Some time during the initial enlargement of the middle layer of cells, cells in the layer immediately above divide horizontally forming the sixth layer (fig. 2 C). Although the rapidly enlarging cells cease to divide in a lateral and vertical plane, they continue to divide in a direction parallel to the longitudinal axis of the leaf at the same rate as the embryonic chlorenchyma cells until cell differentiation begins.

By the time the leaf-blade is about 3.5 mm. wide, all the vascular bundles have been determined, and there are usually four cells in the middle layer between every two bundles so that the pattern is essentially that of the

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Explanation of figure 1

Sections of leaf-blades of *Streptochaeta*. A. Longitudinal section of portion of young leaf of *S. spicata* prior to collapsing of enlarged mesophyll cells. B. Longitudinal section of portion of mature leaf of *S. spicata*. C. Transverse section of portion of mature leaf-blade of *S. spicata*. D. Longitudinal section of portion of mature leaf-blade of *S. Sodiroana*. *ch*—chlorenchyma; *cch*—chlorenchyma connecting enlarged cells; *emc*—enlarged mesophyll cell; *gc*—guard cells; *p*—parenchyma sheath of vascular bundles; *st*—stoma; *ue*—upper epidermis; *vb*—vascular bundles.

mature leaf (fig. 2 C). The middle two members (fig. 2 C, *cch*) of this quartet of cells cease enlarging as soon as they reach the dimensions of a mature chlorenchyma cell, while the outer two (fig. 2 C, *em*) continue to grow. The cytoplasm becomes highly vacuolated in the large cells as they increase in size, whereas it remains dense in the cells of the other layers and of the developing vascular bundles until division ceases and differentiation begins.

Up to the time the blade has emerged from the enclosing sheath of the next lower leaf and has begun to unfold, the enlarged cells of the middle layer are rectangular in shape in longitudinal section and their contents are alive. In surface view they are very long and narrow and are closely packed together. Their nuclei fill the entire width of the cell. At this time, longi-
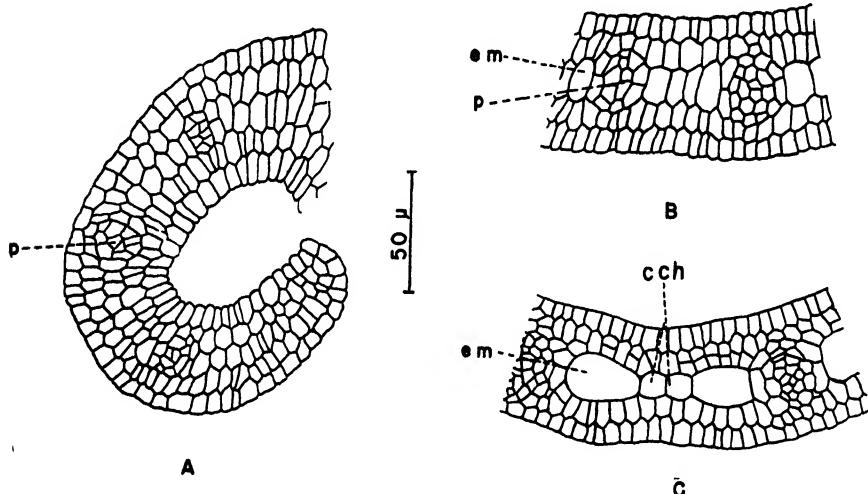
FIG. 2. Transverse sections of young leaves of *S. spicata*. A. Portion of leaf 0.5 mm. broad showing formation of main vascular bundles. B. portion of leaf 2.1 mm. broad showing middle layer of mesophyll beginning to enlarge. C. Portion of leaf 3.5 mm. broad showing adult pattern and formation of sixth layer of cells. *em*—enlarging mesophyll cell; *cch*—chlorenchyma cells connecting two enlarged cells; *p*—procambium.

tudinal sections show small intercellular spaces between enlarged cells where the corners have become rounded and the walls separated. In general the nuclei of these enlarged cells are slightly larger than those of the embryonic chlorenchyma cells, although by the time they have reached their full dimensions, the nuclei of all the mesophyll cells are of approximately the same size.

Just before or soon after unfolding of the leaf-blade, the enlarged cells appear to die, and their walls collapse. The regularity in form of the collapsed cells of the mature leaf seems to indicate that some force was operating on all of them at the same time. The nature of this force has not yet been determined.

The preceding description of the leaf anatomy of *Streptochaeta spicata*

presents a general picture of the bamboo type of leaf pattern. Brandis (1907) in his discussion of bamboo leaves listed several characters which distinguish them from those of other grasses. Among the most important are the following:

1. Full grown leaves flat, with a prominent midrib containing a number of vascular bundles in two lines.

2. Stomata abundant on lower epidermis over chlorophyll parenchyma in longitudinal belts alternating with belts without stomata.

3. Thick-walled lower epidermis with numerous protuberances. Upper epidermis generally smooth.

4. Large apparent cavities in mesophyll between long nerves and bands of bulliform cells, which are filled by flat, thin-walled cells lying upon each other like the leaves of a book. (These are the collapsed enlarged cells). Walls of flat cells collapsing when leaf is full grown leaving large intercellular spaces.

5. Chlorophyll tissue never limited to cylinders around vascular bundles.

6. All chlorophyll cells with peculiar folds in wall dividing each cell into a number of usually incomplete tubes.

It must be emphasized that all these characters are not constant throughout the Bambuseae, nor are all peculiar to the tribe. For instance, in *Chusquea abutifolia* Griseb. there is only one vascular bundle in the midrib. The lower epidermis in *Chusquea sulcata* Swallen is not papillate and the chlorenchyma cells are only slightly cleft. The features which are most constant are the enlarged mesophyll cells, the arrangement of the stomata, the shape of the chlorenchyma cells, the presence of cross veins and in addition, the arrangement of the various tissues. The enlarged cells are usually collapsed, although in some species they remain distended. The deeply cleft chlorenchyma cells are flat in longitudinal sections and are compactly arranged in layers. There are five or six, or sometimes seven, layers of cells in the leaf. Above the enlarged cells there are two or three layers of chlorenchyma and below them one or two layers. Longitudinal bands of collapsed cells alternate laterally with vascular bundles and bands of chlorenchyma one or two cells wide in precisely the same manner as described for *Streptochaeta* (fig. 3 C).

As was mentioned in the beginning, several genera not included in the Bambuseae also possess these same leaf characteristics. These genera (fig. 3) include *Pharus, Leptaspis, Olyra,* and *Diandrolyra* as mentioned by Brandis (1907) and *Lithachne, Mniochloa, Raddia, Pariana,* and possibly *Anomochloa.* All these genera and *Streptochaeta* possess most of the features mentioned by Brandis as being characteristic of bamboo leaves. The large mesophyll cells which may or may not collapse and the spatial arrangement of veins, enlarged cells, and chlorenchyma as described for bamboo and *Streptochaeta* are characteristic of all eight genera. The chlorenchyma is arranged
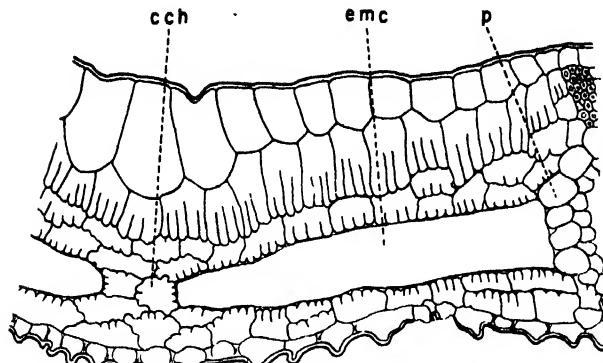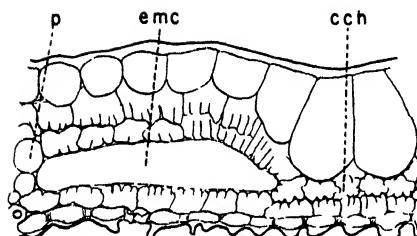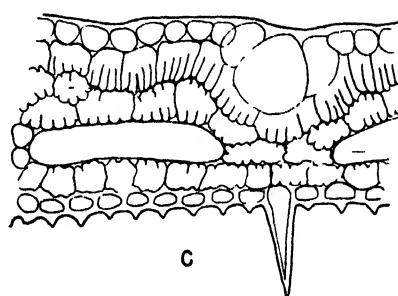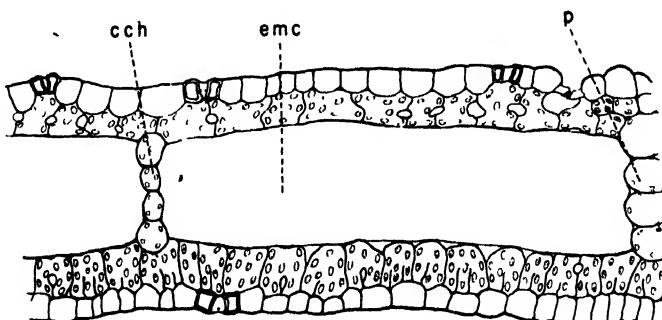
FIG. 3. Transverse sections of portions of leaf blades. A. *Olyra heliconia.* B. *Pariana zingiberina.* C. *Phyllostachys bambusoides* (after Brandis 1907). D. *Pharus parvifolius.* *cch*—chlorenchyma cells connecting enlarged cells; *emc*—enlarged mesophyll cells; *p*—parenchyma sheath of vascular bundles.

in layers above and below the enlarged cells rather than in cylinders around the vascular bundles as it is in many grasses of other tribes. In *Pharus* and *Olyra* the midrib contains two or more vascular bundles. In all the genera the stomata are arranged in longitudinal rows on each side of the vascular bundles and alternate with longitudinal bands of epidermis which are devoid of stomata. The chlorenchyma of *Pharus, Leptaspis,* and *Streptochaeta* differs from the typical bamboo type in that the tissue is less compact, the individual cells are not flat, and their lobes are spread out. The enlarged cells in *Pharus* and *Leptaspis* are of very great size, and the chlorenchyma connecting them is a vertical column one cell wide consisting of three or four very small cells (fig. 3 D). In *Pariana* there may be more than three chlorenchyma cells connecting the enlarged cells (fig. 3 B).

In spite of minor differences in leaf structure between these grasses and the bamboos, the basic pattern is so similar that relationship is suggested. All the genera are of doubtful affinities and have been placed in various' tribes depending on the interpretation of their spikelets. For example: the floral structure of *Streptochaeta* is such that an interpretation of it has been difficult. As a result there have been several explanations and the genus has been classified in almost as many ways as there are interpretations. Originally it was regarded as a special subtribe of the Bambusaceae by Doell (1880), whose opinion is upheld by Mrs. Arber (1929). Celakovsky (1889) and Goebel (1895) considered it very primitive and very close to the ancestral type of grass. Hackel (1887) assigned it to the Oryzeae, while Bentham and Hooker (1883) placed it in the Paniceae. The leaf anatomy, however, favors the opinion of Doell and Arber, and it will be shown in a later publication that the structure of the inflorescence may bear this out. Further study of the inflorescences of the other genera, keeping in mind the possibility of their affinities with the Bambuseae, may prove fruitful.

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MEGASPOROGENESIS AND EMBRYO DEVELOPMENT IN TROPAEOLUM MAJUS L.[1]

RUTH I. WALKER

The mature eight-nucleate female gametophyte of *Tropaeolum majus* L. was described briefly by Woycicki in 1907 (5). Studies on the development of the embryo had been made much earlier. Wilson in 1843 (4) described a root-like process at the base of the bottle-shaped embryo from which two branches arise; one branch perforates the ovule below the micropyle and passes around the ovule in the carpel cavity; the other grows through the funiculus to the placental vascular bundle and downward to the point of union of the carpel and receptacle. Schacht in 1855 (2) reported the formation of two "limbs" from an embryo body; one penetrating the ovary wall, the other growing through the micropyle and into the stylar canal. Observations similar to those of Wilson were later confirmed by Dickson in 1872 (1). He found also that in *T. peregrinum* L. the first branch of the process grew only a short distance in the ovarian cavity and then penetrated the carpel wall, growing obliquely outward and downward, and that in *T. speciosum* Poepp. & Endl. the placental process did not develop.

MATERIALS AND METHODS

Material from two varieties of cultivated nasturtium, the single, climbing type and the ordinary double Golden Gleam, were used in this investigation. Flower buds of various ages, open flowers, and fruits in various stages of development were collected and fixed in Karpechenko's modification of Nawaschin's and formal-acetic-alcohol solutions. The material was embedded in paraffin and sections 10 to 30 μ in thickness were cut, mounted, and stained with either Heidenhain's iron-alum or Delafield's haematoxylin.

MEGAGAMETOPHYTE DEVELOPMENT

The ovary contains from three to five ovules. Each ovule arises as a rounded mass of cells from the surface of the placental tissue at the base of a carpel, and continues to develop by nuclear and cell division. Growth occurs more rapidly on one side of the protuberance than on the other, with the result that the ovule bends toward the stylar end of the ovary (fig. 1).

The inner integument arises from the ovule at a level immediately below the megaspore mother-cell and grows outward and forward around the

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nucellus. The outer integument arises at a position below the inner integument and grows forward shortly after the initial growth of the inner integument. By the time the four megaspores are formed, the inner integument, which is four cells thick, completely surrounds the nucellus and forms a short micropyle. The nucellus persists as a single layer of cells in the micropylar region. Cells at the base of the ovule undergo active mitosis and a massive chalazal region is formed. A single vascular trace is differentiated in the funiculus and forms a direct connection with the vascular system of the ovary.

A hypodermal cell at the apex of the nucellus is differentiated as an archesporial cell at about the time of the initiation of the integuments (fig. 2). This cell functions as the megaspore mother-cell and is easily distinguished from the neighboring nucellar cells by its greater size and more deeply staining properties. The archesporial cell enlarges until it is several times as long as broad at maturity, the nucleus being in the mid-region of the cell (fig. 3).

As a result of the meiotic divisions, a linear row of four megaspores is formed (fig. 4). Ovules were observed in which the four megaspores are not in a linear row (fig. 5). The chalazal spore of the row increases in size and forms the megagametophyte. The remaining three micropylar spores are nearly equal in size and disintegrate during the development of the megagametophyte. Many ovules were seen in which the nuclei of the micropylar megaspores had undergone division before disintegration, as is shown in figures 6, 7, and 10.

The cytoplasm of the megaspore is highly vacuolate. The vacuoles coalesce as the cell grows, resulting in a large central vacuole at the time of the completion of the first nuclear division (fig. 6). The micropylar nucleus divides, the plane of division being at right angles to the long axis of the megagametophyte; the chalazal nucleus divides also, the plane of division being transverse or parallel with the long axis of the megagametophyte. The nuclei resulting from these divisions are embedded in cytoplasm (fig. 7). One further division occurs (fig. 8) and four nuclei are formed at each end of the cell (fig. 9); this is followed by cell wall formation. A typical seven-celled megagametophyte is formed, consisting of three antipodal cells at the chalazal end, two synergids and the egg of the micropylar end, and a large primary endosperm cell in the central region (fig. 10). The polar nuclei migrate toward each other, come in contact anterior to the mid-region of the cell and fuse to form a large primary endosperm nucleus (fig. 11). The antipodal cells are small and may assume varying positions (figs. 10, 11, 12). They distintegrate at about the time of fertilization.

The egg apparatus consists of a large pear-shaped egg and two elongated synergids. During the earlier stages of development of a synergid, the
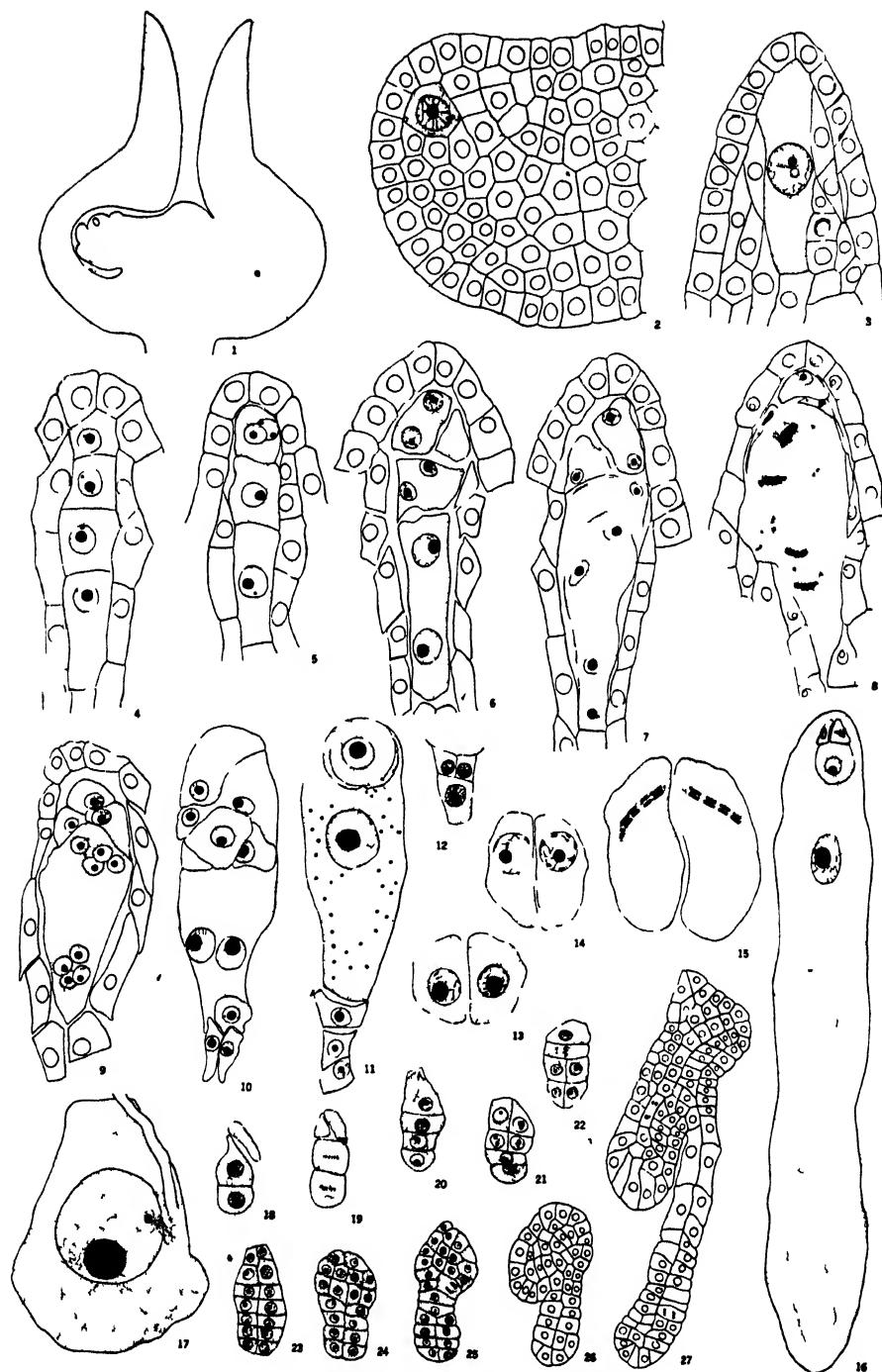
nucleus is centrally located and surrounded by finely vacuolate cytoplasm (fig. 13). A large vacuole appears at the micropylar end of the cell which later disappears and a new vacuole is formed at the base of the cell (fig. 14). Synergid nuclei appear to be in the process of mitosis although none was observed in which division was completed (fig. 15). The egg cell grows and the large basal portion extends into the megagametophyte beyond the synergids. The egg nucleus is located toward the basal portion of the cell and is embedded in cytoplasm. Starch grains are abundant in the cytoplasm surrounding the egg nucleus and in the young endosperm mother cell but none are present in either the antipodals or the synergids (fig. 11).

FERTILIZATION

Fertilization takes place from 12 to 19 hours after pollination. In this process the pollen tube grows through the micropyle and enters the megagametophyte between the two synergids. The synergids are not destroyed by the entrance of the pollen tube and may persist until the four-celled embryo stage (fig. 20) No early stages of the union of one of the male nuclei with the egg nucleus were observed. Figure 17 shows a young zygote nucleus in which the chromatic network of the male and female nuclei are indistinguishable from one another. Two nucleoli are now visible in the zygote nucleus, the larger, the nucleolus of the female gamete, and the smaller, the nucleolus of the male gamete. The second male nucleus fuses with the endosperm. Division of the endosperm nucleus occurs shortly after fertilization. Often two or four endosperm nuclei are found before the first division of the zygote has occurred.

Explanation of figures 1–27

FIG. 1 Longitudinal section of ovary showing young ovule. × 30. FIG. 2. Young ovule with hypodermal archesporial cell. × 375. FIG. 3. Nucellus, megaspore mother-cell. × 375. FIG. 4. Linear row of four megaspores. × 375. FIG. 5. T-shape tetrad of megaspores. × 375. FIGS. 6–15. Stages in the development of the megagametophyte. FIG. 6. Two nucleate megagametophyte and disintegrating megaspores. × 375. FIG. 7. Four nucleate megagametophyte and disintegrating megaspores. × 375. FIG. 8. Third nuclear division. × 375. FIG. 9. Eight-nucleate megagametophyte. × 375. FIG. 10. Young megagametophyte before fusion of the polar nuclei. × 375. FIG. 11. Older megagametophyte, polar nuclei fused. × 375. FIG. 12. Antipodals of megagametophyte. × 375. FIG. 13. Young synergids. × 600. FIG. 14. Older synergids. × 600. FIG. 15. Still older synergids at time of fertilization × 600. FIG. 16. Mature megagametophyte. × 140. FIG 17. Young zygote showing remains of pollen tube. × 600. FIG. 18. Two-celled proembryo, remains of one synergid. × 140. FIG. 19. Two-celled proembryo, nuclei in process of division. × 140. FIG. 20. Four-celled proembryo, remains of one synergid present. × 140. FIG. 21. Vertical division of haustoria cells. × 140. FIG. 22. Vertical division of apical and suspensor cells. × 140. FIG. 23. Further development of embryo, suspensor and haustoria. × 140. FIG. 24. The same. × 140. FIG. 25. The same. × 140. FIG. 26. Initiation of the first haustorium. × 140. FIG. 27. Older embryo, suspensor and haustoria. × 140.

EMBRYO DEVELOPMENT

The zygote divides transversely to form a two-celled proembryo, the two cells approximately equal in size (fig. 18). Both cells divide to produce a linear row of four cells (figs. 19, 20). The embryo develops from the apical cell of the tier, the suspensor is formed from the subapical, and the haustoria develop from the remaining two cells. Further divisions occur in the four cells, the plane of division being first vertical and then horizontal (figs. 21, 22), so that a 14- to 16-celled proembryo is formed (fig. 23).

The basal cells of the proembryo continue to multiply more rapidly than the apical and suspensor cells, forming a wedge-shaped mass (figs, 24, 25, 26, 27). Peripheral cells of this mass adjacent to the suspensor and on the
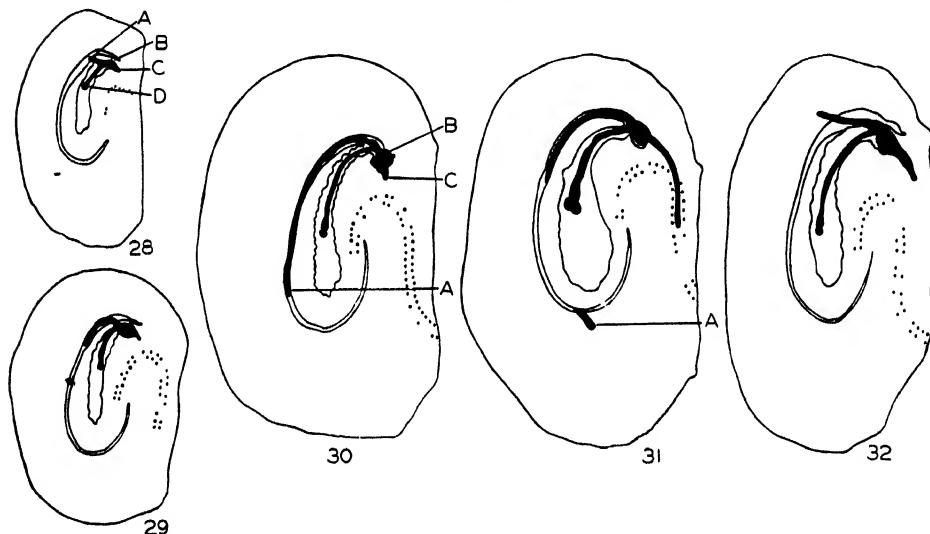
FIGS. 28–32. × 25. FIG. 28. Longitudinal section of carpel showing outline drawing of embryo, suspensor and haustoria. FIG. 29. The same showing first haustorium in carpel cavity. FIG. 30. The same showing older stage of first haustorium in carpel cavity. FIG. 31. The same showing first haustorium in carpel wall. FIG. 32. The same showing growth of first haustorium in carpel wall.

side opposite the funiculus become actively meristematic and a root-like haustorium is initiated which penetrates the integument near the micropyle and grows around the ovule in the carpel cavity and into the carpel wall (figs. 28, 29, 30, 31). Haustoria were observed penetrating the carpel wall opposite the micropyle (fig. 32). This was reported by Dickson as the characteristic condition for *T. speciosum* and *T. peregrinum*.

Early in the development of the haustorium, the apical cells of the structure are elongate in shape and contain dense cytoplasm with large nuclei (figs. 33, 34, 35). As growth continues, they become from three to seven

times as long as wide. The cytoplasm is coarsely vacuolate and giant nuclei appear to be undergoing mitosis although no division figures were observed. Nuclei similar to these were described by Woycicki (6) in 1907.

Shortly after the initiation of the first haustorium, a second protuberance arises from the peripheral cells nearest the funiculus (fig. 27). Multiplication of the peripheral cells continues until a wedge-shaped structure is formed. Figure 36 is a longitudinal section of such a structure as indicated at "C" in figure 28. The innermost portion of this process is composed of large, mature cells, rectangular in section and with coarsely vacuolate cytoplasm; the cells at the apical portion of the structure are small with nuclei embedded in dense cytoplasm. As a result of the embryonic activity and elongation of the apical cells (fig. 37), the second haustorium grows through the nucellus, the integument and the funiculus (figs. 28, 29, 30), and upon reaching the vascular bundle of the placenta, grows down along the side of the vascular bundle of the raphe or between the component elements of the vascular bundle (fig. 31). The haustorium pushes aside the cells of the nucellus and integument until it comes in contact with the cells of the vascular tissue of the funiculus. During this development the peripheral cells of the basal portion of the placental haustorium grow also, especially in length, giving a finger-like appearance (figs. 30 "B," 38). This elongation may be as much as three or four times the original size. A large basal vacuole develops in each cell and the nucleus becomes embedded in cytoplasm in the anterior region of the cell.

The cells of the funiculus above the region where the vascular bundle curves and enters the ovule are filled with starch grains in the early stages of the development of the haustorium. In older stages, fewer starch grains are present. It is assumed that this starch is the main source of food for the developing haustorium until it reaches the vascular bundle, where nutrients may be absorbed directly from this tissue. As the apex of the placental haustorium grows forward through the raphe (fig. 39), it does not necessarily grow directly downward but may grow in a curving manner (fig. 40), thus increasing the absorbing area of the structure.

The growth of the embryo and suspensor occurs concurrently with the development of the two haustoria (fig. 28 "D"). The cells of the embryo continue to divide and a spherical mass of cells is formed (fig. 41) which later becomes somewhat pear-shaped, the apical portion being broader than the base. Cell divisons occur also in the supensor, the plane of division usually being transverse except in those cells adjacent to the embryo in which it is both longitudinal and transverse A collar-like structure, composed of elongated cells, is differentiated at the basal end of the suspensor (fig. 42). The position of these cells in relation to endosperm probably facilitates the absorption of the food materials (figs. 44, 45). The elongation of the suspensor pushes the embryo into the endosperm.
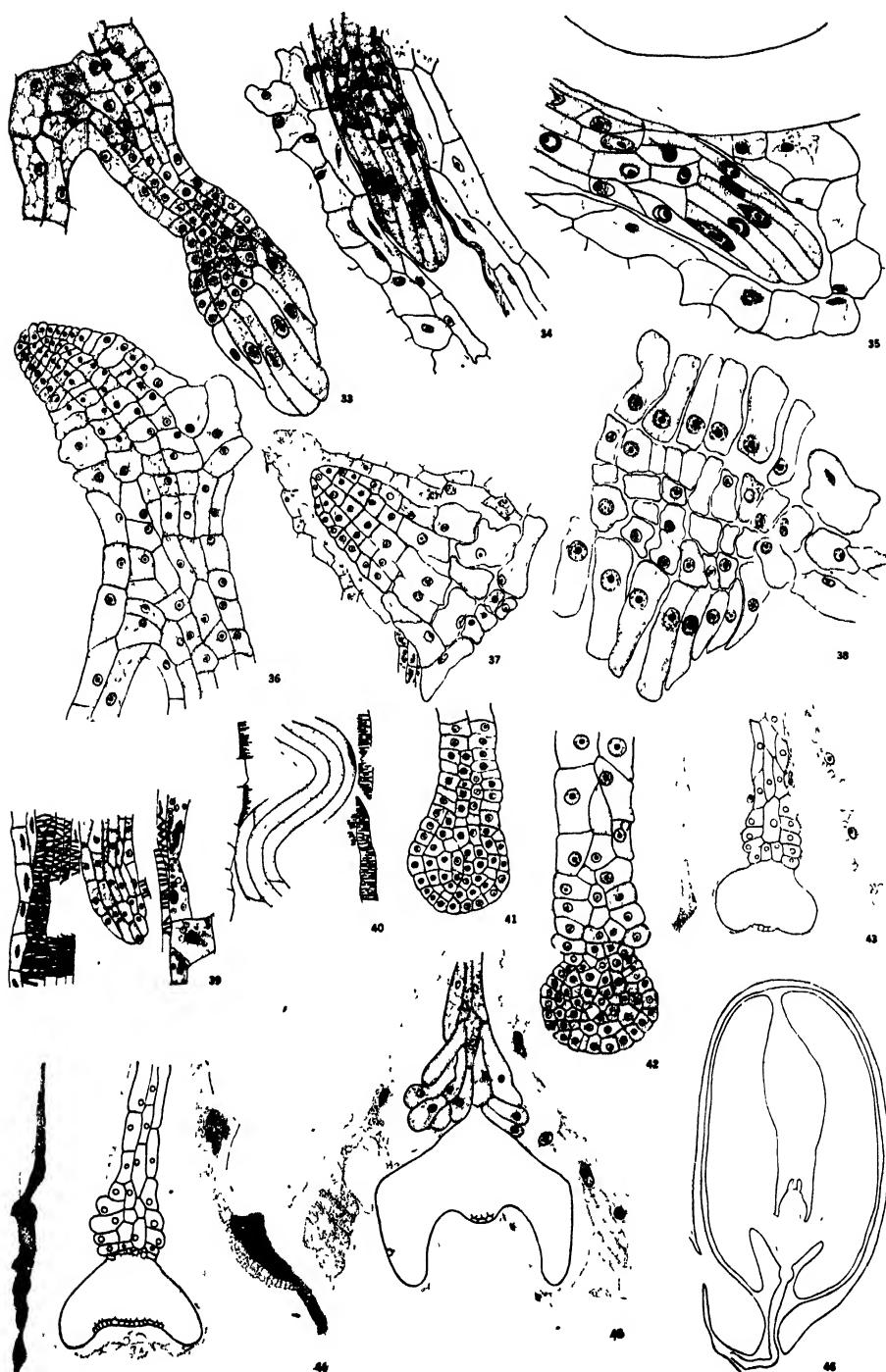
The cotyledons appear as two lateral outgrowths at the apex of the embryo opposite each other (fig. 43). The epicotyl develops as a small outgrowth between the cotyledons at the apex of the embryo. Basal cells of the embryo later form the hypocotyl (figs. 44, 45) The cotyledons elongate rapidly, forming two larger fleshy structures at maturity. A mature embryo showing the differentiation of vascular strands is shown in figure 46.

The growth of the embryo is accompanied by increase in size of the growing seed. The cells of the integuments and of the nucellus adjacent to the chalazal region multiply rapidly; the plane of division of the integumentary cells is such that it does not increase materially in thickness. The nucellus, which is wide at the base and tapers toward the micropylar end of the ovule, consists of an outer layer of small, densely staining cells and an inner layer of large, irregularly shaped cells, with central vacules and little cytoplasm. These become disorganized and are absorbed by the endosperm.

The development of the embryo and surrounding tissue is dependent upon the nutrient materials brought into the ovule by the vascular tissue from the adjacent parts of the plant. In the early stages of growth some of the nutrients are stored as starch in the cells of the funiculus just above the entrance of the vascular bundle into the ovule. The greatest amount of starch is observed in these cells when the embryo is about five days old. As the embryo and haustoria mature, the food is digested and absorbed by the peripheral cells. During this time the endosperm develops slowly; some of the nuclei are found in a thin layer of cytoplasm adjacent to the embryo, while others are embedded in dense cytoplasm contiguous to the nucellus (fig. 43). The endosperm remains free-nucleate throughout its entire development. No trace of the endosperm or nucellus is found at maturity and the seed consists of the embryo surrounded by the seed coat through which extends the slender, thread-like haustorium.

Explanation of figures 33–46

Fig. 33. Detailed drawing of apical portion of young haustorium indicated at ''A'' in figure 28. × 140. Fig. 34. The same for older haustorium in carpel cavity as indicated at ''A'' in figure 30. × 90. Fig. 35. The same for older haustorium in carpel wall as indicated at ''A'' in figure 31. × 90. Fig. 36. Detailed drawing of placental haustorium as indicated at ''C'' in figure 28. × 140. Fig. 37. The same for apical portion of an older placental haustorium indicated at ''C'' in figure 30. × 140. Fig. 38. The same for the basal portion of older placental haustorium as indicated at ''B'' in figure 30. × 140. Fig. 39. Portion of mature placental haustorium adjacent to vascular tissue. × 90. Fig. 40. Curving growth of placental haustorium. × 90. Fig. 41. Detailed drawing of embryo and portion of suspensor as indicated at ''D'' in figure 28. × 140. Fig. 42. Detailed drawing of somewhat older embryo and portion of suspensor. × 140. Fig. 43. Embryo showing initiation of cotyledons and epicotyl and their relation to the endosperm. × 50. Fig. 44. Somewhat later stage showing embryo and endosperm. × 50. Fig. 45. Still later stage of embryo and endosperm. × 50. Fig. 46. Longitudinal section through maturing ovule showing embryo, suspensor and integuments. × 90.

Abortive ovules commonly occur in *Tropaeolum,* especially in the semi-double Golden Gleam variety. Longitudinal sections of ovaries show that all the ovules of the ovary mature about the same time. Four days after pollination many of the young seeds possess well developed embryos; in others, syngamy has not occurred and the megagametophytes show signs of disintegration. The megagametophytes are much elongated; the egg and primary endosperm nuclei are highly vacuolate and the cytoplasm of the primary endosperm cell has almost disappeared. Nucellar cells are likewise in a state of disorganization. The walls of the cells adjacent to the megagametophyte are thick and have a callus-like appearance. With the increase in age of the ovary, the abortive ovule may enlarge for a time but much less rapidly than the fertilized ovule. Growth finally ceases and the ovule collapses and becomes shrivelled in appearance.

SUMMARY

A single hypodermal cell is differentiated as an archesporial cell in *Tropaeolum majus.* This functions directly as the megaspore mother-cell.

A linear row of four megaspores is formed. Occasionally megaspores may be in a T-shaped arrangement.

The chalazal spore develops into an eight-nucleate, seven-celled megagametophyte; the other megaspores disintegrate.

Syngamy occurs 12–19 hours after pollination.

The synergids are not destroyed as a result of the entrance of the pollen tube and may persist for several days, then disintegrate.

The antipodals disappear before syngamy.

The zygote by transverse divisions forms a row of four cells. The embryo develops from the apical cell of the row, the suspensor from the subapical cell, and the haustoria from the two remaining micropylar cells.

The haustorium which develops first penetrates the integuments below the micropyle and grows around the ovule in the carpel cavity and into the carpel wall opposite the chalazal region of the ovule. The placental haustorium arises later, and grows through the integument and placenta to the point of entry of the vascular bundle of the raphe. The haustoria supply food to the developing embryo.

The endosperm is free-nucleate and is absorbed by the embryo during the course of its development.

The embryo develops in a typical manner having the lateral cotyledons, a terminal epicotyl, and a hypocotyl.

Some ovules may abort through the failure of syngamy.

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HIPPEASTRUM SOLANDRIFLORUM: ITS CHROMOSOMES

J. T. BALDWIN, JR. AND BERNICE M. SPEESE

Hippeastrum solandriflorum (Lindl.) Herb. is found from Brazil to Colombia and the Guianas (Macbride 1936) and, according to the interpretation of Herbert (1837), occurs in several varieties. It was introduced into cultivation in 1820 (Baker 1888) and has been used to a considerable extent in wide crosses, as have many representatives of the genus. Indeed, Bailey (1938) states: "Most of the hippeastrums in cultivation are probably hybrids."

E. P. Killip identified *Baldwin 3099*, now in the U. S. National Herbarium, as *H. solandriflorum*: in flower, September 26, 1943, Braco, Rio Arinos, Matto Grosso, Brazil. The specimen rather well matches *plate 3771* in *Curtis's botanical magazine* for 1840; that illustration is based on a collection made by Schomburgk in British Guiana. In May, 1946, a bulb from the Rio Arinos produced a flowering plant (fig. 1) under greenhouse conditions in Virginia, and, after the scape had withered away, gave rise in September, 1946, to a pair of narrow leaves edged with red. The mature capsule was reddish. Seedlings were grown from that plant.

The senior author found *H. solandriflorum* at three places in Matto Grosso: at Braco, in a sandy area near the Rio Arinos and where grasses, sedges, and trees (among others *Hancornia speciosa* Gomes) are scattered; shortly downstream from Salto Bello on the Rio Sacre; and near Rosario Oéste, across the southern rim of the Amazon Basin, in sandy soil that becomes closely packed when dry and where *Arachis* sp. grows.[1] The hippeastrum was in blossom in September and October, 1943. The flowers were extremely showy. No leaves were present. Populations were large. In two years (1942–1944) of almost continuous travel throughout the Amazonian

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[1] It is worthy of record that in September and October, 1943, *Arachis* was observed in flower on the outskirts of Rosario Oéste and also where the road leading northward from that town crosses the rim of the Amazon Basin. The plants of that rim seem in great measure different from those constituting the flora of the regions to the north and south of the rim. On December 3, 1943, in Matto Grosso, flowering specimens were obtained for *A. marginata* Gardn. at Tres Lagóas (*Baldwin 3139*), for *A. Diogoi* Hoehne at Campo Grande (*Baldwin 3140*), for *A. marginata* Gardn. at Corumbá (*Baldwin 3141*): identifications were made by F. C. Hoehne and E. P. Killip. The two collections designated *A. marginata* are of at least varietal difference. The specimens are in the U. S. National Herbarium. The subterranean fruit of *Arachis*, like the pronounced underground development of *Macrosiphonia longiflora* (Desf.) Muell. Arg., of *Anacardium pumilum* St. Hil., and of many other plants of that region, is apparently an adaptation to dryness. Fire may well have served as an agent of selection.
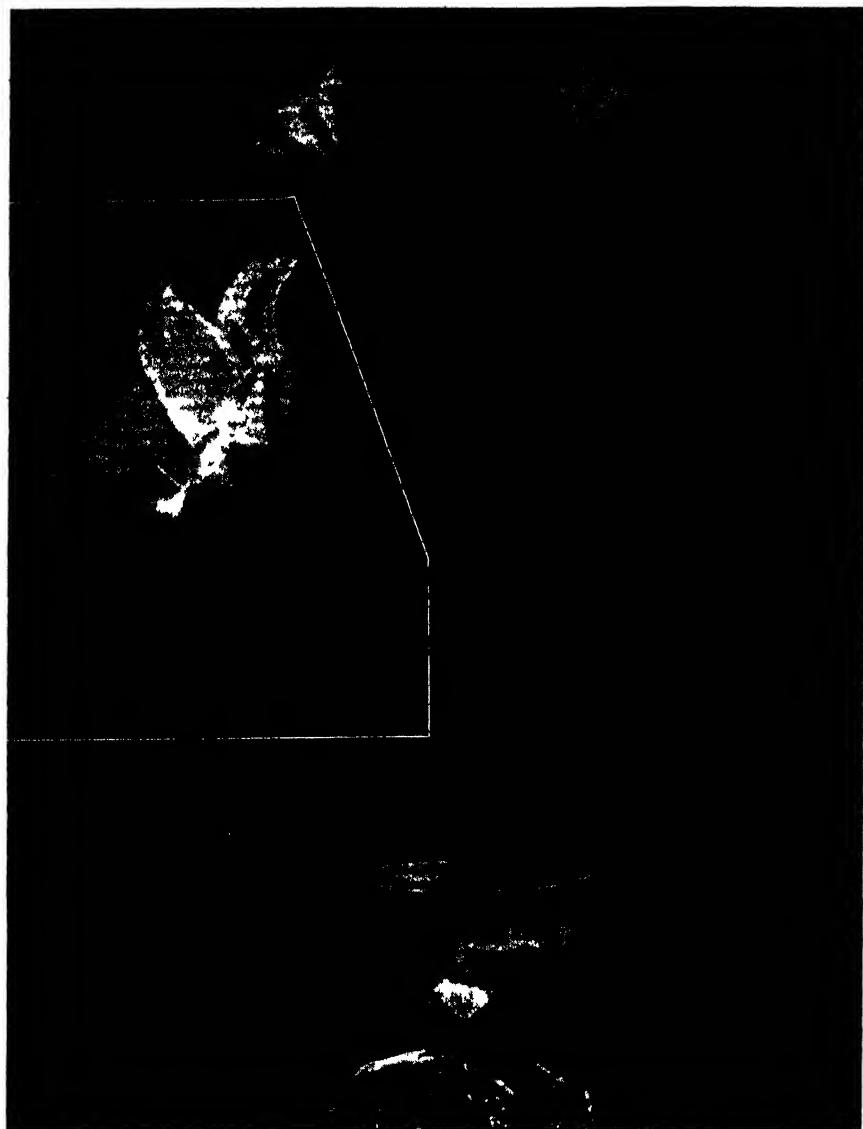
FIG. 1. *Hippeastrum solandriflorum* (Lindl) Herb.; inset photographed slightly under natural size and reduced about ⅓ in reproduction.

region and in some of the country immediately south of the Basin, no more impressive assemblages of bulbous plants than these were seen, unless, perhaps, the stands of *Zephyranthes* along the Rio Cuyabá be excepted.

The perianth of *H. solandriflorum* is greenish yellow and reaches a length of eight inches. Two or four flowers are borne on a scape, which may attain
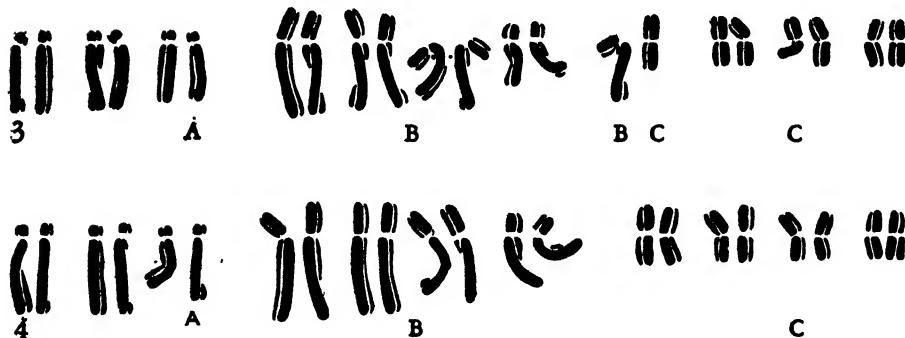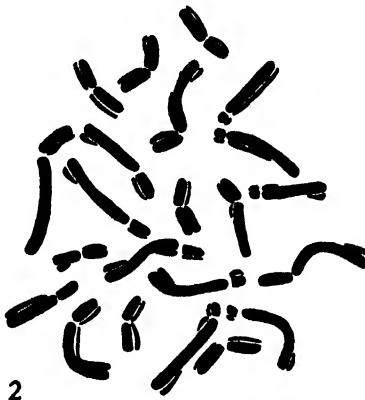
FIGS. 2–4. Chromosomes of *Hippeastrum solandriflorum*, ×1100. FIG. 2. Metaphase from root of Rio Arinos plant with chromosomes distributed in drawing: $2n = 22$. FIGS. 3, 4. Alignment of metaphase chromosomes of two seedlings of Rio Arinos plant. Figure 3 has unequal pair of chromosomes present, as in parent. Figure 4 has no unequal pair of chromosomes.

a height of two and a half feet. The flowers are at first erect but with maturity turn downward. The spathe soon wilts. Leaves are produced later and may reach a length of one and a half feet (Baker 1888): those of our plant are in November, 1946, not yet six inches long.

Because the Rio Arinos collection had not been "contaminated" through cultivation, it was decided to investigate the chromosomes of that plant and of some of the seedlings raised from it. Preparations were made by the junior

author. Roots were treated with colchicine, fixed in Carnoy's solution (3 absolute alcohol : 1 chloroform : 1 glacial acetic acid), and smeared in propiocarmine.

H. solandriflorum from the Rio Arinos has a 2n-number of 22 (fig. 2). The plant investigated has one unequal pair of chromosomes. Five of the seedlings grown from that plant have been cytologically studied. Four of them are like the parent, with three pairs of chromosomes in the A group (which has a short short arm), four pairs in the B group (with a longer short arm), three pairs of approximately isobrachial chromosomes in the C group, and an odd pair with one chromosome of the B type and one of the C (fig. 3). Classification into groups is arbitrary but convenient. Obviously, chromosomes in different roots and at different mitotic stages exhibit varying degrees of contraction when treated with colchicine. The fifth seedling has no odd pair of chromosomes (fig. 4) : three pairs are in group A, four in group B, four in group C. If additional seedlings were studied, individuals with five pairs of B-type chromosomes would probably be encountered. Similarly: if the cytological structure of the Rio Arinos population of *H. solandriflorum* were to be analyzed with respect to the classification of chromosomes given here, it would be expected that a great—but unpredictable—percentage of the individuals would have a pair of chromosomes made up of B and C members.

Not many reports of chromosome numbers for *Hippeastrum* have been made:

	2n	
H. rutilum	44	Satô (1938)
H. rutilum fulgidum	24(?)	Heitz (1926)
H. vittatum Herb.	46	Nagao & Takusagawa (1932)
"	44	Inariyama (1937)
"	44	Satô (1938)
H. hybridum	44	Inariyama (1937)

Inariyama (1937) and Satô (1938) suggested that their plants were tetraploid; the latter author classified the chromosomes as to types. *H. solandriflorum* from the Rio Arinos is accordingly diploid. A program of hybridization based upon a cytological knowledge of the various species of *Hippeastrum* should be most productive both of scientific and of horticultural results.

SUMMARY

Populations of *Hippeastrum solandriflorum* (Lindl.) Herb. were observed at three places in Matto Grosso, Brazil. A single plant from the Rio Arinos was cytologically studied. It is diploid with a 2n-number of 22 chromosomes, which can, on the basis of centromere location, be arbitrarily grouped into classes A, B, and C; one chromosome pair, with B and C members, is unequal.

Four of the seedlings grown from the wild plant and investigated were found to be like the parent in having a heteromorphic pair of chromosomes. The fifth seedling examined has no unequal pair of chromosomes: a C chromosome has been substituted for the odd B. If additional seedlings were to be studied, it is likely that individuals with a B chromosome replacing the odd C would be discovered. Accordingly, a considerable number of the plants in the Rio Arinos population should have an unequal pair of chromosomes made up of B and C members.

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ALASKA AND YUKON SPECIES OF RUBUS SUBGENUS CYLACTIS FOCKE

J. P. ANDERSON

In studying the genus *Rubus* for the purpose of writing the manuscript for the Flora of Alaska and adjacent parts of Canada the writer became aware of the differences in treatment of certain groups within the genus by our very best authorities. This is especially true of the species closely related to *R. articus* L. Dr. L. H. Bailey,[1] the leading American authority on the genus, believes that the true *R. articus* does not occur in North America and includes all American forms of this type in *R. acaulis* Michx. Dr. Eric Hultén[2] of Sweden, the world's leading authority on arctic and boreal plants in general, includes most of the collections in *R. articus* but also recognizes *R. acaulis* and hybrids between the two. Bailey describes a new species, *R. alaskensis,* which Hultén regards as a hybrid between *R. articus* and *R. stellatus* Smith. After a study of more than three score sheets in my collections, mostly from Alaska but some from Yukon and other parts of northern Canada, I have come to the following conclusions.

RUBUS ARTICUS L. does occur in North America and *R. acaulis* Michx. should be regarded as a synonym or at best only a forma or subspecies of it. Many intermediate forms occur. If *R. acaulis* is regarded as a separate entity these intermediate forms may be regarded as hybrids, but it seems doubtful if the differences between the two types are sufficient to warrant the separation. Many other species of plants show a similar wide variation.

RUBUS ALASKENSIS is a good species. When Dr. Bailey described this species he had before him three of my collections. No. 2A128 collected on a muskeag 11 miles northwest of Juneau, no. 955 collected at Matanuska, and no. 1378 collected in the valley of the Little Susitna River a short distance above where its canyon emerges from the foothills of the Talkeetna Mountains onto the level stretches of the Susitna-Matanuska valley. Since then I have made additional collections, no. 6981 at the type locality, no. 6989 several miles up the same valley, no. 7208 at Curry, no. 7606 at Talkeetna. It is most common at Talkeetna. Hultén accounts for the large size of this form by hybrid vigor. I do not think that this is at all probable. *Rubus stellatus* is common in the Pacific coastal areas of Alaska but of very scattered occurrence in the interior. *R. articus* is common in the interior but reaches

[1] The Genus *Rubus* in North America. Gent. Herb. 5: 24–30, 34–38, 40.

[2] Flora of Alaska and Yukon. Lunds Univ. Assrk. NF. Avd. 2. 42¹: 992–997, 1005–1007.

the Pacific coast only at the head of deeply penetrating inlets. Occasional crosses of *R. arcticus* and *stellatus* are found that show no noticeable increase in vigor. It therefore seems unreasonable to assume that hybrids of these two species which are usually less than 20 cm. and often less than 10 cm. tall, and both wholly herbaceous, should in a few instances give rise to a form usually woody at the base and up to 52 cm. tall. When I collected the type number I was so impressed with the size, vigor, and some of the other characteristics of the plants that I thought it might be a cross of the large species with perennial canes, *Rubus spectabilis* Pursh, which was growing near by, and *R. stellatus*. The distribution of *R. alaskensis* is also unfavorable to the hybrid hypothesis. All the specimens except 2A128 were collected in a strip along or near the Alaska Railroad less than 100 miles long in south central Alaska. No *R. arcticus* is known within 50 miles of the place where 2A128 was collected. The leaves and general appearance of *R. alaskensis* are distinctive as compared to its assumed parents. In my opinion no. 955 cited by Bailey is *R. alaskensis* × *arcticus*. All the other numbers cited are typical *Rubus alaskensis*.

Regarding the other two species of the subgenus *Cylactis* in Alaska and Yukon there is no controversy. I collected *Rubus pubescens* Raf. at Watson Lake in Yukon territory but it is not known to reach Alaska. *Rubus pedatus* Smith is common in the Pacific coast regions of Alaska. The five species of the subgenus occurring in Alaska and Yukon may be keyed as follows.

1A. Flowers pink or red.
  1B. Leaves 3-lobed.   *R. stellatus.*
  2B. Leaves trifoliate.
    1C. Stems 3–25 cm. tall, entirely herbaceous and slender.   *R. arcticus.*
    2C. Stems 20–50 cm. tall, stout and often woody.   *R. alaskensis.*
2A. Flowers white.
  1D. Stems slender and creeping, rooting at the nodes, leaves appearing
      5-foliate.   *R. pedatus.*
  2D. Producing runners but flowering stem arising from the crown, leaves
      3-foliate.   *R. pubescens.*

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PLANTS OF IMPORTANCE IN THE BREEDING OF ANOPHELES ALBIMANUS WIED. IN PANAMA[1]

Samuel Lewis Meyer

In Panama, as elsewhere in the Caribbean region, the most important vector of malaria is *Anopheles albimanus* Wied., a "white-footed" mosquito of the group Nyssorhynchus. It is a medium-sized, grayish mosquito which is common in the lowlands of the Caribbean region. Its distribution ranges from Sinaloa, Mexico, to Guayaquil, Ecuador; along the Gulf coast from the lower Rio Grande Valley, Texas, south and east to Surinam; and in the West Indies, except Martinique, St. Lucia, Grenada, and Trinidad (Ross & Roberts 1943). The adult is a strong flier and bites man freely.

Anopheles albimanus breeds in a variety of habitats, including fresh to brackish water, but more abundantly in full sunlight in bodies of water where there are floating mats of vegetation. Within the vegetation mats, the larvae are protected from such natural enemies as surface-feeding fish of various kinds and, at the same time, find an abundant supply of food (Rozeboom 1941). Gatún Lake, the Gatún River, and the Chagres River contain extensive "floating islands" of vegetation which serve as breeding grounds for *Anopheles albimanus*.

In 1944–45, the writer served with the Army School of Malariology in the Canal Zone. At that time, the opportunity was presented to observe the composition of the vegetation mats in the Gatún River and the Chagres River, and the relative importance of the various aquatic plants composing the mats in the breeding of *Anopheles albimanus*. The results of those observations are reported here. The nomenclature used with reference to mosquitoes follows Komp (1942).

It is a well known fact that *Anopheles albimanus* breeds in various types of floating aquatic vegetation in the Canal Zone. Curry (1934) pointed out that *Eichhornia crassipes* (Mart.) Solms, *E. azurea* (Swartz) Kunth, and *Pistia stratiotes* L. were present in abundance in Gatún Lake but stated that the aquatic plants of greatest importance in anopheline breeding were *Utricularia mixta* Barnh. and several species of *Chara* L. The latter plants were said to provide optimum conditions for the breeding of anopheline mosquitoes that require sunlit waters. This was especially true of mosquitoes of the Nyssorhynchus group to which *Anopheles albimanus* belongs.

[1] Contributions from the Botanical Laboratory, The University of Tennessee, N. Ser. No. 95.

The observations of Curry (1934) with reference to *Utricularia* and *Chara* are of particular interest. Matheson (1930) had suggested that *Utricularia* deserved more consideration as a possible agent in mosquito control since larvae were captured in the bladders. Curry, however, pointed out that the surface-feeding and floating habits of anopheline larvae would render them very little susceptible to capture by the submerged traps of ·*Utricularia*. Matheson also reviewed the conflicting literature with reference to the influence of *Chara* on the development of mosquito larvae. Some workers had indicated a marked inhibitory effect; others observed that the Characeae had little, if any, influence on mosquito breeding. Experimental results of Matheson and Hinman (1929) indicated that certain vigorously growing species of *Chara* in some way prevented the development of larvae, while Matheson (1930) observed that oviposition did not take place where the growth of *Chara* was vigorous. Curry (1934) stated that in tropical and subtropical regions the Characeae favored the development of mosquitoes and that the aquatic flora of *Chara* and *Utricularia* in Gatún Lake provided a breeding place for vast numbers of anophelines. More recently, Rozeboom (1941) reported the Characeae, *Utricularia*, and *Najas* to be especially favorable for the development of *Anopheles albimanus*.

The great vegetation mats in the Chagres and the Gatún Rivers are composed of a relatively small number of species of vascular plants.

Eichhornia crassipes (Mart.) Solms, the common water-hyacinth of South America which has become naturalized in Panama as in some parts of the United States, and *E. azureus* (Swartz) Kunth, the native water-hyacinth of Central America, are found in great abundance. Barber and Hayne (1925) have found anopheline breeding among *E. crassipes* in the southern United States while Eyles and Robertson (1944) reported the same species to be associated with *Anopheles quadrimaculatus* Say in the southeastern United States.

Pistia stratiotes L., the water lettuce, is also present in the vegetation mats. It is widely distributed in tropical regions and seems to be extremely favorable to insect life (Dunn 1934). Though undoubtedly much more intimately associated with the breeding of *Anopheles triannulatus* Neiva & Pinto (Curry 1932; Komp 1942), larvae of *Anopheles albimanus* have been collected from the crowns of *Pistia stratiotes* in the Canal Zone (Zetek 1920). It should be noted that *Anopheles triannulatus*, formerly known as *A. bachmanni* Petrocchi, is of no importance as a vector of malaria in Panama. In Brazil, Bachmann (1921) found that mosquitoes appeared to avoid *Pistia stratiotes*.

Cabomba aquatica Aubl., listed by Standley (1928) as the only Central American species of fanwort, is sometimes found in the mats.

Jussiaea natans H.B.K. is a common floating plant of the Chagres River

and Gatún Lake. It is sometimes found in the vegetation mats. In Panama, larvae of *Anopheles triannulatus* are found along the floating stems (Komp 1942). In the southeastern United States, *Anopheles quadrimaculatus* has been found in association with *Jussiaea diffusa* Forsk. and *J. grandiflora* Michx. (Eyles & Robertson 1944).

The water ferns, *Azolla* Lam., possibly more than one species, and *Salvinia natans* L., are often present, sometimes in sufficient quantity to cover considerable areas. Matheson (1930) called attention to the conflicting observations made on the influence of various species of *Azolla* on mosquito breeding. Some investigators have found species of *Azolla* to be effective

Fig. 1. A view of the Gatún River showing a "floating island" of vegetation, composed principally of *Najas* L.

in preventing anopheline breeding while others have observed a favorable influence.

The most important component of the vegetation mats in the Chagres and Gatún Rivers is *Najas*. It is present in such quantity as to cover extensive areas of water surface (fig. 1). One collection from Gatún Lake and another from the Chagres River have been identified by Clausen (1946) as *Najas arguta* H.B.K., a South American species. The collection from the Chagres River was made by Lt. Harold Trapido, of the Gorgas Memorial Laboratory. Clausen states that "according to Lt. Trapido *N. arguta* is a dominant feature of the aquatic flora of the Chagres River and Gatún Lake, also it is the principal breeding place of *Anopheles albimanus*." Whether the *Najas* in the Gatún River is the same species and whether more than

one species is present in Gatún Lake, the Gatún River, and the Chagres River is not yet known. There is no doubt but that at the present time the most important plant in the Canal Zone from the standpoint of its relation to the breeding of the malaria vector is *Najas*. The "floating islands" of that plant provide a breeding place for incalculable thousands of anopheline mosquitoes.

No mention has been made of *Utricularia* or members of the Characeae. The writer had no opportunity to study the vegetation of Gatún Lake. It is not known whether the Utriculariaceae and Characeae were ever present in such quantity as to be of significance in anopheline breeding in the Chagres and the Gatún Rivers. If that were ever the case, they have now almost completely disappeared and in those areas would certainly no longer be a factor.

It is important to note that Curry (1934) does not mention *Najas* as an important component of the vegetation mats in Gatún Lake. Clausen (1946) cites Lt. Trapido who terms *Najas arguta* H.B.K. the "dominant feature of the aquatic flora" of that part of the Panama Canal system. This indicates that great changes in the composition of the flora of Gatún Lake and, possibly also, the Gatún and Chagres Rivers may have taken place. It is of interest to note in that connection that Curry called attention to the fact that water lettuce had flourished in Gatún Lake for several years and then had "spontaneously and for some unknown reason" almost disappeared.

A factor in producing changes in the observable vegetation pattern of Gatún Lake may have been the erection of Madden Dam across the upper Chagres, a possibility which was recognized and suggested by Curry at the time of its construction. The dam has provided less fluctuation in the water level of the lake and thus there may be less *Chara* and *Utricularia* exposed. At the same time, the reduction in the fluctuation of the water level has apparently provided excellent conditions for the growth of *Najas*.

These observations on the composition of the vegetation mats of two of the rivers of Panama illustrate not only the important role which aquatic vegetation may play in mosquito breeding but also show how the botanist may serve with the entomologist, the parasitologist, and the engineer in attacking the malaria problem.

SUMMARY

The most important vector of malaria in Panama, as in the entire Caribbean region, is *Anopheles albimanus* Wied. It breeds in a variety of habitats, including fresh to brackish water, but most abundantly in full sunlight in bodies of water where there are mats of floating vegetation. The most important elements in the composition of the vegetation mats in the Gatún and Chagres Rivers are: *Najas* sp., possibly *N. arguta* H.B.K., *Eichhornia*

crassipes (Mart.) Solms, *E. azureus* (Swartz) Kunth, *Cabomba aquatica* Aubl., *Jussiaea natans* H.B.K., *Salvinia natans* L., *Azolla* sp., and *Pistia stratiotes* L. Of these, the most important is *Najas*.

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TORREYA

SHORT ARTICLE

Noteworthy Records from the Local Area. During the past few years some very noteworthy botanizing has been carried on by two young botanists in the local area. It has been my privilege to examine most of these collections, and numerous records worthy of publication have come to light. Mr. Harry Ahles has collected over 1175 botanical specimens in Pelham Bay Park in Bronx County, New York, and in some areas of Westchester County. Mr. Joseph V. Monachino has continued collecting in various parts of New York and New Jersey. The following are a few of the most interesting plants found by these collectors, and a corrected identification of one of my own numbers.

Agastache scrophulariaefolia (Willd.) Kuntze—several colonies on a hillside facing east, Van Cortlandt Park, Bronx Co., N. Y., August 12, 1945, *J. V. Monachino 133*. Norman Taylor's "Flora of the Vicinity of New York" says that this species occurs "on the north shore of Long Island and formerly on Staten Island, thence increasing, but never common, northward." This is the first Bronx County specimen in the Local Herbarium at the New York Botanical Garden.

Centaurea vochinensis Bernh.—in a waste lot, North Plainfield, Somerset Co., N. J., August 18, 1934, *H. N. Moldenke 8226*. This collection was originally distributed by me as *C. nigra* L., but Mr. Monachino has kindly pointed out the differences between these two very similar species. *C. nigra* also grows in our local area and is far more common. Gray's "Manual" (ed. 7) states that *C. vochinensis* is found locally from New England to Ontario. Britton & Brown's "Illustrated Flora" (ed. 2) gives its distribution as "Ontario to Massachusetts and southern New York," and Taylor says that it is "rare" from Ontario to southern New York. The New Jersey record therefore appears to be well worth recording.

Cynanchum nigrum (L.) Pers.—along roadsides, section 13, Pelham Bay Park, Bronx Co., N. Y., May 24, 1946, *H. Ahles 276*. The distribution of this European species is given by Gray as from Massachusetts and Vermont to Pennsylvania and Ohio, while Britton & Brown say "Massachusetts to Pennsylvania and Ohio and in British Columbia"; Taylor says "Mass. to Pa. and Ohio."

Elaeagnus umbellata Thunb.—abundantly naturalized in thickets, section 28, Pelham Bay Park, Bronx Co., N. Y., July 11, 1946, *H. Ahles 537*. This Asiatic species is not listed by Gray, Taylor, or Britton & Brown, but is widely naturalized now in our area. I have previously recorded it from various parts of New Jersey.

Euonymus europaeus L.—a single colony found on a rock ledge, in partial shade, section 8, Pelham Bay Park, Bronx Co., N. Y., May 31, 1946, *H. Ahles 305*. Gray says that this European plant "occasionally escapes from cultivation in the Atlantic States," and Taylor says it is "rather rare." The present record seems worth noting.

Lychnis alba var. *colorata* Lange—found along roadsides, section 2, Pelham Bay Park, Bronx Co., N. Y., June 7, 1946, *H. Ahles 376*. This pink-colored form of the

species is not identified in any of our current manuals, although it is listed in most European floras.

Persicaria longiseta (De Bruyn)Moldenke—common on farmland and along roadsides, near Carmel, Cumberland Co., N. J., August 25, 1946, *J. V. Monachino 160*. This east Asiatic ladysthumb, easily the handsomest *en masse* in our area, is not listed in any current manual, but (as was pointed out by me in TORREYA **41**: 201–203) is spreading rapidly along the eastern seaboard.

Plantago Rugelii var. *asperula* Farwell—along shore, section 19, Pelham Bay Park, Bronx Co., N. Y., July 11, 1946, *H. Ahles 549*. Deam's "Flora of Indiana" is practically the only one of our current manuals to list this hairy variety of Rugel's plantain.

Rosa setigera Michx.—along the bridle path, in a naturalized state, Pelham Bay Park, Bronx Co., N. Y., July 19, 1946, *H. Ahles 638*. This prairie species is recorded by Gray as escaped from cultivation only in Connecticut, while Britton & Brown say "escaped from cultivation in Connecticut, New Jersey, and Virginia." The present New York record seems worthy of note.

Thalictrum aquilegifolium L.—one plant found, at edge of woods, section 11, Pelham Bay Park, Bronx Co., N. Y., May 31, 1946, *H. Ahles 291*. This species is not listed in any current manual as having escaped anywhere in North America.

Trifolium pratense var. *albiflorum* Pluskal—in a meadow, Pelham Bay Park, Bronx Co., N. Y., July 23, 1946, *H. Ahles 662*. This albino form is not identified in any of the current popular manuals of American flora, but is listed in European works.

Vicia sepium L.—in thin woods, quite common, Pelham Bay Park, Bronx Co., N. Y., June 15, 1946, *H. Ahles 187*. Gray gives the American distribution of this European species as "locally, Me. to Ont."

Vicia villosa Roth—in meadow, section 5, Pelham Bay Park, Bronx Co., New York, July 11, 1946, *H. Ahles 175*. Gray says for this species "inclined to persist or escape," and Britton & Brown say "locally spontaneous." The present definite record seems worth noting.—HAROLD N. MOLDENKE.

PROCEEDINGS OF THE CLUB

Minutes of the Meeting of January 15, 1947. Dr. Simpson called the regular afternoon meeting of the Torrey Botanical Club to order at 3: 45 p.m. at the New York Botanical Garden. Seventeen members and friends were present.

No business was transacted and the meeting was turned over to Dr. Helen Purdy Beale who spoke on: "Some properties of Tobacco Mosaic Virus." Dr. Beale's talk was illustrated with lantern slides and was followed by much interesting discussion.

The meeting was adjourned at 5: 00 p.m. and was followed by refreshments served by members of the Garden's staff.

Respectfully submitted,
LIBERO AJELLO,
Recording Secretary

Minutes of the Meeting of February 5, 1947. The regular evening meeting of the Torrey Botanical Club, held in conjunction with the Brooklyn Botanic Garden's Plant Science Lectures, was opened by Dr. Shull at 8: 20 p.m. in the research building of the Brooklyn Botanic Garden. Thirty-one persons attended the meeting.

The minutes of the previous meeting were not read. Seven annual members and five associate members were presented to the Club for approval and all were elected unanimously.

No further business was transacted and the meeting was turned over to Dr. L. M. Black, who presented a very thorough and stimulating account of his research work on "Plant Tumors Induced by Viruses." A lively discussion followed the presentation of this fine paper.

The meeting was adjourned at 9: 45 p.m.

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Respectfully submitted,

LIBERO AJELLO,

Recording Secretary

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Minutes of the Meeting of February 19, 1947. The regular evening meeting of the Torrey Botanical Club was opened by Dr. Shull at 8: 00 p.m. at Columbia University with 24 members and friends present. The minutes of the meetings of January 15th and February 5th were approved as read.

The memorial to the late Dr. R. A. Harper, prepared by Dr. B. O. Dodge, Dr. H. W. Rickett and Dr. J. S. Karling was read before the members. Dr. Zimmerman moved that the Club send a vote of thanks and its compliments to the committee for their work. The motion was seconded and carried unanimously.

Dr. Shull read a letter from the Adirondack Club requesting the Torrey Botanical Club to cooperate in its efforts to prevent the granting of mining rights and the opening of recreational areas in the State's forest preserve. All members were urged to write to Senator Phiny W. Williamson protesting such a move.

No further business was transacted and the meeting was turned over to Dr. Ralph C. H. Siu, who spoke of his work at the Philadelphia Quartermaster Depot on the: "Fundamental Aspects of the Microbiological Degradation of Cotton." The paper was ably presented and provoked a lengthy discussion. The author's abstract follows:

As a result of intensive work on the problem by the Quartermaster Corps, the N.D.R.C. and collaborating institutions, over 10,000 cultures of microorganisms have been isolated from deteriorated cotton fabrics. Of these, about 200 cultures have been shown to possess cellulolytic ability. It is doubtful, however, whether all these are of considerable importance under field conditions. In attacking the cotton fiber the fungal hypha penetrates the fiber wall into the lumen where it proliferates and digests the cotton fiber outwards. Bacteria, on the other hand, adhere to the outer surface and pit their way inwards. In both cases, the attack appears to be a highly localized affair with degradation occurring only at the point of the fiber in immediate contact with the microorganism.

The organisms secrete cellulose-digesting enzymes. So far, these have been classified into 2 classes, viz.: (a) cellulase, which converts cellulose into cellobiose and (b) cellobiase, which converts cellobiose into glucose. Cell-free preparations of these cellulose-degrading enzymes have been made and their properties extensively studied.

There are four general lines of approach to the development of preventive methods, viz.: (1) physical preventation of organism from making contact with the cellulose molecule, as illustrated by the resistance of resin-impregnated cloth, (2) cell toxicants, as exemplified by fungicides, (3) specific enzyme inhibitors which exist today as a theoretical possibility, and (4) chemical modification of the cellulose molecule, which appears to be very promising for future exploitation.

The meeting was adjourned at 9: 15 p.m. and was followed by tea prepared by members of the Columbia Botany Department.

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Respectfully submitted,

LIBERO AJELLO,

Recording Secretary

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Field Trip Reports

September 22. Butler to Pompton Plains, N. J. "Old Mother Nature sure did her part. The day was fine, the display of gentians was *much* the finest I ever saw in N. J., the orchids (*Spiranthes cernuua*) were there, literally in thousands, and the grapes were fully ripe and delicious." Leader, H. B. Gordon.

September 28. The New York Botanical Garden. "Because of the long stretch of dry weather, we found rather poor picking for the fleshy fungi. Special attention was given to a parasitic fungus, *Pseudonectria pachysandricola* on *Pachysandra*, which Dr. Dodge investigated and described. It was quite destructive to this host until Dr. Dodge worked out methods of controlling it." Attendance 16. Leaders, Fred J. Seaver and B. O. Dodge.

September 29. Dock Watch Hollow, N. J. An interesting host of woody and herbaceous plants was observed in this ramble through the Watchung Mountains hereabouts. No specialties were reported. Attendance 31. Leader, Harold N. Moldenke.

September 29. Princeton Jct. vicinity, N. J. This walk was a circular that included pond, stream, field, and woodland. The latter was observed in different aspects as we passed along Grover's Pond, crossed the Millstone River and returned along Plainsboro Pond. A comparative list of species would have been interesting for there are striking variations. Of course, with these leaders, many interesting bird experiences were adduced. Attendance 14, plus three who overshot the meeting place but caught up with the leaders in time to be counted. Leaders, Peg and Charles Rogers.

October 5. Southward from High Point, N. J. The Appalachian Trail survey and check for the Guide included the walk along the Kittatinny Ridge, descent to Lake Rutherford, and a visit to the shelters along this portion of the Trail. Some good views but no plant specialties reported. Attendance 9. Leader, L. E. Hand.

October 6. From Liberty Corner, N. J., westward on the Appalachian Trail. This list has not been compiled as yet. The Trail largely follows roadways here and some new species for the Trail List are expected. Attendance 11. Leader, G. G. Nearing.

October 6. Richmond, Staten Island. Although a bus strike prevented the leader's arrival, three people did make it and report a nice walk through old fields and over the salt marshes.

October 12. Woodlands, N. Y. "The ominous clouds distracted me to the point of overlooking a rich stand of *Solidago odora* I had meant to call attention to. We did notice a lot of *Solidago speciosa*, some *S. flexicaulis* and plenty of the more common goldenrods—*S. canadensis*, *S. rugosa*, *S. bicolor*, *S. caesia*, *S. graminifolia*. The various bushes provided a patriotic display of red, white, and blue berries, while the trees laid particular stress on the golden hues. The outstanding feature of the trip was an exceptionally profuse growth of *Baeomyces roseus* with exceptionally large pink fruiting bodies. An eight-year-old had the time of his life looking at the lichens through a lens. Said he, 'It is quite something to see so many things under the magnifying glass.' From his questions and remarks I am hopeful the little boy is a nature student in the making." Attendance 6. Leader, Alexandra Kalmykow.

October 13. Cheesequake Park, N. J. This walk over varied terrain featured the seeding and fruiting of plants as well as other aspects of dormancy in anticipation of the winter season. A great variety of edible fruits was sampled, most satisfying being the persimmons. A preliminary list of 400 seed plants and ferns so far determined from the Park is available from the field chairman upon request. Attendance 16. Leader, John A. Small.

OCTOBER 19. APPALACHIAN TRAIL BETWEEN CATFISH POND GAP AND MILLBROOK ROAD, N. J. This survey with its list of plants about completes the project from the Hudson River to the Delaware. There are a few places where one trip did not quite join where another left off. There are some five miles over Mt. Tammany and down to Columbia where the Trail has been relocated since our survey of the Dunnfield Creek route. Compilation is not completed yet. Has it been worth while? Shall we start along beyond the rivers? Attendance 12. Leader, Louis E. Hand.

NOVEMBER 10. LAKEWOOD, N. J. A very pleasant experience in the pine barrens in autumn. Lichen studies were added to the interesting notes on the higher plants. Attendance 17. Leader, James Murphy.

NEW NOTES

Alice Eastwood Herbarium Accessions. Miss Alice Eastwood, 88-year old Curator of Botany of the California Academy of Sciences, reports that the Academy Herbarium, named in her honor, has received 9180 mounted specimens during the past year. Of these, 2978 accessions were by gift, 4722 by exchange with other herbariums, 1480 were by exploration. The Alice Eastwood Herbarium now contains a total of 334,000 mounted botanical specimens.

Wanted, Back numbers of the BULLETIN. The supply of back numbers of certain issues of the BULLETIN of the Torrey Botanical Club remaining in the possession of the Club are very low, and we are completely out of a few numbers. Unfortunately some of these numbers are of recent date and are in demand by foreign libraries which could not get the BULLETIN during the war, or which may have been destroyed. If any members have copies of the following numbers which they would be willing to donate to the Club we shall be very glad to receive them:

Vol. 67 (1940), Nos. 1, 2, 5, 6.
Vol. 68 (1941), Nos. 1, 2, 3, 4.
Vol. 70 (1943), Nos. 1, 2, 3.
Vol. 71 (1944), Nos. 1, 2.

They should be sent to Harold H. Clum, Business Manager, Hunter College, 695 Park Ave., New York 21, N. Y.

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Lazella Schwarten

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POLYPLOID MITOSIS IN THE NORMAL DEVELOPMENT OF MIMOSA PUDICA[1]

E. R. WITKUS AND C. A. BERGER

In seedlings of *Allium cepa* it has recently been found (Berger & Witkus 1946) that certain cells become tetraploid and divide once as tetraploids in the course of normal development. The tetraploid cells were found in the cortex of the cotyledon and of the intermediate region between root and shoot. They were never found in the roots of *Allium*. Cytological study revealed that the polyploid condition arose by a double reproduction of the chromosomes in the resting nucleus.

A similar process has recently been found in the seedling roots of the sensitive plant, *Mimosa pudica,* and is here reported. The diploid chromosome-number of this plant is 48. The chromosomes show little variation in size and are small, the largest being about $2.8\,\mu$ in length (figs. 1–4).

Mitoses first appeared in seedlings 2 mm. in length, earlier growth apparently being due to expansion through intake of water. Tetraploid divisions were abundant at the beginning of mitotic activity in the root meristem. An average of 40 4n mitoses were found in 2 mm. seedlings, the extremes being

TABLE 1. *Frequency of 4n divisions.*

	Length of seedlings in mm.									
	1	2	3	4	5	6	7	8	9	10
	0	20	20	60	16	15	2	1	0	0
	0	32	27	25	20	3	1	0	0	0
	0	38	33	125	40	0	1	0	0	0
No. of 4n	0	30	120	111	25	7	0	3	0	0
cells in	0	30	25	170	20	1	2	0	0	0
division	0	36	45	63	17	6	8	2	1	0
	0	35	83	88	20	5	3	4	0	0
	0	27	79	150	20	12	5	3	0	0
	0	39	64	55	33	6	0	2	0	0
	0	110	50	89	20	10	0	1	0	0
Average	0	40	55	94	23	6	2	2	.1	0

20 and 110. The frequency of 4n divisions increases to a maximum in seedlings 4 mm. in length and then decreases. The average number of 4n divisions found in 4 mm. seedlings was 94 with extremes of 25 and 170. In seedlings more than 10 mm. in length no polyploid divisions were found, although diploid divisions were very abundant. Up to the present no polyploid divi-

sions have been found in root-tips taken from mature plants, although polyploid cells are undoubtedly present. The data on length of seedling and frequency of tetraploid divisions is given in table 1.

The polyploid divisions were found only in large cells of the periblem, usually in the two outermost cell-layers of this tissue. In a 2-mm. seedling the first 290 μ are exclusively root-cap, diploid divisions begin at this point and extend backward to a distance of about 800 μ from the tip. The tetraploid divisions are found in the region from 600 to 800 μ back from the tip. In a 4 mm. seedling the root-cap occupies the first 450 μ and the tetraploid divisions begin at a distance of 840 μ and extend backward to a point 1500 μ from the tip.

The polyploid divisions were all tetraploid and all showed a paired arrangement of the chromosomes which was especially noticeable at prophase (figs. 5–9). The close pairing is a clear indication that the tetraploid condition arose by a double reproduction of the chromosomes in the preceding resting stage and that this is the first mitosis since the polyploid condition was attained. Since only tetraploid cells with paired chromosomes were found it is probable that these cells undergo only one division as tetraploids before becoming differentiated. As has been shown in *Spinacia* (Berger 1941) the close pairing of chromosomes is lost during anaphase and telophase and if such polyploid cells undergo a second division the chromosomes are completely unpaired at prophase and metaphase. No such unpaired polyploid divisions were found in any of the *Mimosa* seedlings examined. Since these tetraploid divisions are found in the roots of very young seedlings (2 mm. in length) when the majority of the cells are undergoing their first division, it is probable that the polyploid condition arose during the early embryology of the plant.

DISCUSSION

The type of polyploidy here described is similar in many points to that found in *Allium* and differs markedly from polysomaty as described in *Spinacia*. In *Mimosa* as in *Allium* the tetraploid cells are found at a definite time and place and thus constitute a localized factor in the developmental pattern. In both of these plants the tetroploid cells divide only once before they become differentiated to a degree that usually excludes further mitosis. In *Spinacia*, cells which become tetraploid may undergo more than one division as tetraploids and some pass through further double chromosome reproduction becoming 8-ploid or even 16-ploid. Furthermore, in *Spinacia*, the formation of polyploid cells and their division continue as long as the root-tips are actively growing, i.e., throughout the whole indeterminate growth period of the adult plant, while in *Mimosa* and *Allium* the process is limited to a definite period during germination.

In all three plants the polyploid condition arises in the same way by a
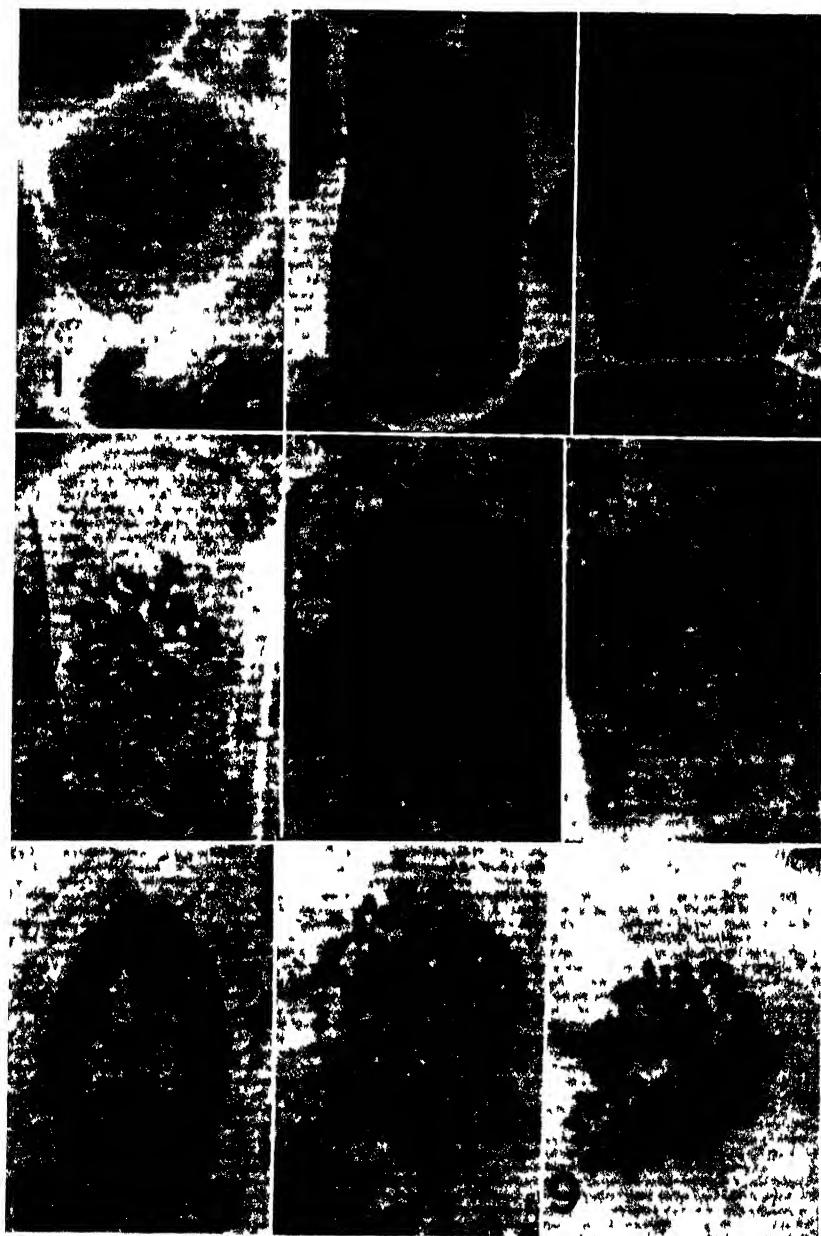
FIGS. 1–9. Photomicrographs of aceto orcein smear preparations of seedlings of *Mimosa pudica*. Magnification ×1500. FIGS. 1–4. Diploid metaphases, showing somatic pairing. FIG. 5 Early prophase of a 4n division, SA region undivided. FIGS. 6, 7. Later prophases of 4n divisions, showing pairing. FIGS. 8, 9. Metaphases of tetraploid divisions.

process of double chromosome reproduction in the resting nucleus. An interesting point of difference is found in the behavior of the spindle-attachment regions of the chromosomes of newly formed polyploid cells. In *Allium* the pairs of chromosomes are held together by a single undivided SA-region until late prometaphase, forming tetrachromosomes. At metaphase two successive divisions of the SA-region occur and anaphase separation is normal. In *Spinacia* the pairs of sister chromosomes are held together by a pronounced relational coiling although their SA-regions are separate throughout most or all of prophase. In *Mimosa* the pairs of sister chromosomes are joined by an undivided SA-region in very early prophase (fig. 5) but not in later phophase (figs. 6, 7). They are not held together by any pronounced relational coiling but are arranged in evenly spaced pairs in which only an occasional half-twist is found.

Very frequently diploid metaphase figures in *Mimosa* showed the chromosomes in pairs. Such somatic pairing in the diploid cells of certain plants has been previously reported in the literature but these cases were not clearly demonstrated. In *Mimosa* the pairing is unmistakable and much too frequent to be a chance phenomenon (figs. 1–4). The cause of this pairing is wholly unknown. The fact of somatic pairing of chromosomes in the Diptera has been well known since the work of Metz (1916). In the mosquito, *Culex pipiens,* Grell (1946) traces the somatic pairing found at metaphase to an active coming together of homologous chromosomes at the preceding anaphase and explains this movement on Darlingtonian lines by assuming that the anaphase chromosomes are single structures. Her evidence for this view is considerable. One of the more weighty points of evidence is the prophase pairing, which is so close in *Culex* that most figures appear to contain the haploid number of prophase chromosomes. This theory however cannot be applied to the somatic pairing in *Mimosa,* since in this plant the diploid prophases show no pairing whatever, although it is very clear in diploid metaphases and in tetraploid prophases.

Preliminary investigations revealed that tetraploid divisions also occur in the large cortex cells of the shoot of young seedlings of *Mimosa*. A more thorough study of this region is in progress.

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CHAPTALIA NUTANS AND C. INTEGRIFOLIA: THEIR CHROMOSOMES

J. T. BALDWIN, JR. AND BERNICE M. SPEESE

Chaptalia Vent. is an American genus of the Compositae. Burkart's (1944) treatment of the genus suggests that it consists of 40–50 species. The northernmost, and type, species is *C. tomentosa* Vent., with occurrence in the moist pinelands of the Coastal Plain from North Carolina to Florida and to Texas (Small 1933). The genus is variously represented from our Southwest—Texas, New Mexico, and Arizona—to Argentina and Chile.

Incidental to field work in Brazil for the U. S. Department of Agriculture the senior author collected fruiting specimens of two species of *Chaptalia*. The specimens have been deposited in the herbaria of the U. S. National Museum, U. S. National Arboretum, New York Botanical Garden, and the Instituto Agronômico do Norte. The collections were kindly identified by S. F. Blake as follows:

C. NUTANS (L.) Polak.
Baldwin 3116. Salto Bello, Rio Sacre, Matto Grosso. October 21, 1943. *Baldwin 3138.* Sitio de Ponte (south of Rosario Oéste), Matto Grosso. November 2, 1943. *Baldwin 4733.* Weed in Jardim Botanico, Rio de Janeiro. November 18, 1943. *Baldwin 4734.* Seringal Monte Alegre (about 40 miles south of Rio Branco), Acre Territory. January 6, 1944.

C. INTEGRIFOLIA (Cass.) Baker
Baldwin 3121. Sucurima (near Agua Limpa), Matto Grosso. October 30, 1943. *Baldwin 3133.* Parecis, Matto Grosso. October 31, 1943. *Baldwin 3135.* Corrego do Amolar (northwest of Rosario Oéste and in Amazon Basin), Matto Grosso. November 1, 1943. *Baldwin 3136.* Tambidouri (near Rosario Oéste), Matto Grosso. November 1, 1943.

According to Burkart (1944) *C. nutans* occurs from Mexico to the Río de la Plata and is the commonest species in the genus; by means of var. *texana* (Greene) Burk., *C. nutans* extends into Texas. That author relegates *C. integrifolia* to synonymy under *C. integerrima* (Vell.) Burk., a species of Brazil, Uruguay, Paraguay, Argentina, Bolivia, and Peru.

Seed of *C. nutans* from weeds (collected November 18, 1943) growing in the Jardim Botanico at Rio de Janeiro and of *C. integrifolia* from plants (collected November 10, 1943) on the airfield at Campo Grande, Matto Grosso, were planted in Virginia in August, 1944. They are perennials. Both species are easy to grow. Under greenhouse conditions flowering and fruiting have been erratic and at various seasons. *C. integrifolia* usually comes into flower somewhat before *C. nutans* does. Where collected in Brazil, as indicated above, these species flower and fruit from about mid-October to mid-January, and thus at the end of the dry season and at the beginning of the rainy. Both species are inconspicuous in flower, but in fruit they are readily

seen, even at a distance from a moving truck, by which means trips were made from Cuyabá, Matto Grosso, north and westward to the Rio Papageio and north and eastward to the Rio Arinos. The usually solitary fruiting head of these species, borne on a scape that rises about a foot above a basal rosette (fig. 1), is suggestive of *Taraxacum,* or perhaps more so of *Tragopogon.* Transition from flowering to fruiting is rapid and, in the greenhouse, may take place overnight.
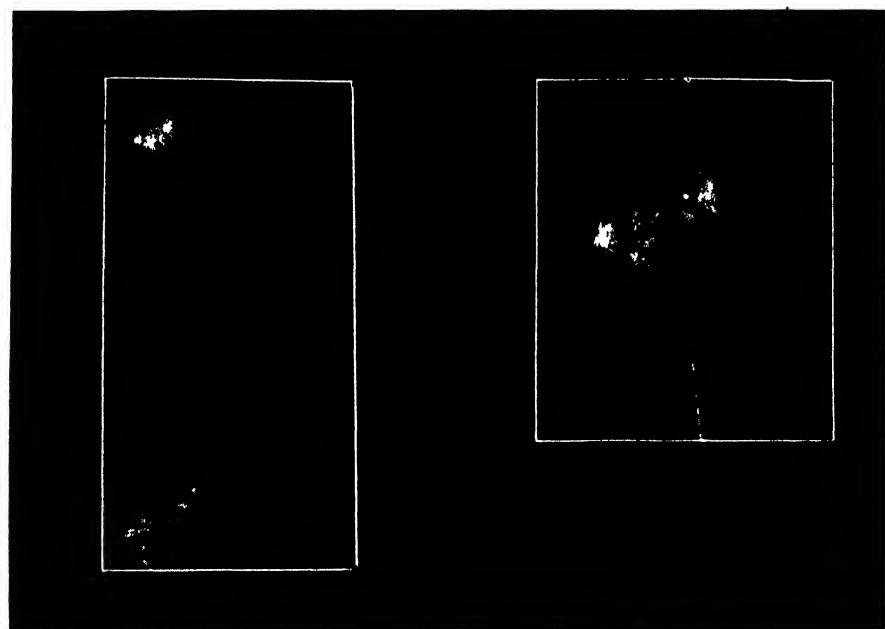
FIG. 1. Fruiting plant of *Chaptalia nutans* as grown under greenhouse conditions in Virginia. Fruiting head on right reproduced just under natural size. Photographed by Conrad Yocum.

Both these species of *Chaptalia* grow in places that become exceedingly dry and where grasses and other herbaceous plants are sparsely distributed and where stunted trees may be scattered. However, both species were never seen together. *C. integrifolia* seems to show a preference for the red-soiled areas of the dry parts of Matto Grosso.[1]

Burkart (1944) recorded cytological observations on *C. exscapa* (Pers.) Baker and *C. piloselloides* (Vahl) Baker. The chromosomes in sectioned root-

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[1] It was in those same areas that especially large villages of termite "houses"— some of them rising to a height of several feet—were observed. When those structures crumble, new ecological situations are available to plants, and, because of that, "islands" of vegetation different from the surrounding grasses and other herbaceous plants are frequently developed. *Smilax* is often one of the first invaders.

tips were small and numerous—for a metaphase of the first species more than 60 chromosomes were determined; for various metaphases of the second, counts of 54, 60, 49–54, 58–60, and 51–54 were arrived at.

C. nutans and *C. integrifolia* proved to be extremely difficult for cytological study. Many smears of leaves, flowers, and roots were prepared, both with and without colchicine treatment. Precise chromosomal analysis was not possible, and differing counts were made. Finally, the junior author obtained entirely satisfactory smears of roots by resorting to a modification of the paradichlorobenzene technique of Meyer (1945):[2] root-tips were placed in a saturated solution of paradichlorobenzene for 2 hours, rinsed in water, fixed in Carnoy's fluid (3 absolute alcohol : 1 chloroform : 1 glacial acetic acid), hydrolyzed in acid alcohol (1 concentrated hydrochloric acid : 1 absolute alcohol) for several minutes, stained in propio-carmine for 12 hours, hydrolyzed again for several minutes, transferred momentarily into Carnoy's solution, and smeared in propio-carmine.

The $2n$-number of chromosomes for both species is 48 (figs. 2, 3). Centromeres are median, submedian, or, for some of the smaller chromosomes, subterminal. Both species have 8 chromosomes that are strikingly longer than the other members of the complement. Of the 8 long chromosomes 4 have submedian centromeres, and 4 have median. These latter 4 may readily be misinterpreted and so give a basis for inaccurate counts. This observed morphological similarity is suggestive of tetraploidy.
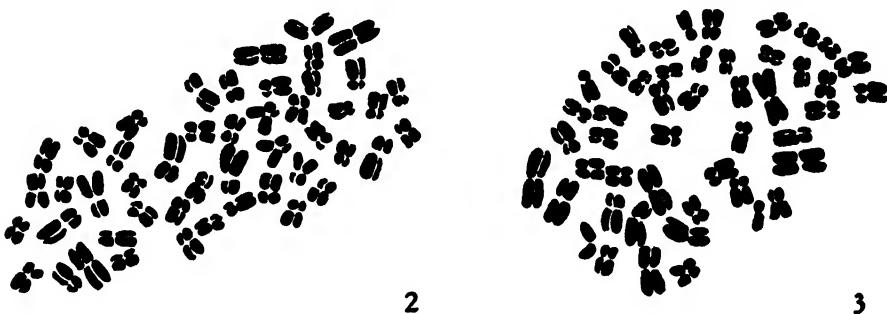
FIGS. 2, 3. Metaphase chromosomes from root smears of *Chaptalia.*× 2200. FIG. 2. *C. integrifolia.* FIG. 3. *C. nutans.* The $2n$-number in both species is 48, with 8 strikingly long chromosomes.

An inclination in recent years to consider that plants are likely to be weeds because of polyploidy is apt to be without adequate basis. *Chaptalia,* for instance, seems not to support such a view. *C. nutans,* the only weed in the genus (Burkart 1944), may, of course, be polyploid, but it has the same chromosome number that *C. integrifolia* does. The two species investigated by Burkart will perhaps likewise be found to have $2n$ of 48, or approximately that number.

[2] Meyer developed the technique for study of guayule chromosomes.

By some writers *Chaptalia* has been confused to some extent with *Gerbera* Gronov., a genus of Asia and Africa. Burkart (1944) discusses the likenesses and differences of these genera. Of interest in this regard are the following chromosome reports for *Gerbera:*

		n	$2n$	
G. Anandria Schultz	Makomanai, Japan		46	Matsuura & Sutô 1935)
"	Hokkaidô, Japan	23		Kishimoto (1940)
"	Kôbe, Japan	23		"
G. integripetala Hay	Formosa	23		"
G. Jamesoni Bolus.	Cultivated	25	50	"
" var.				
hybrida Hort.	Cultivated	25	50	Bowden (1945)

Conceivably, further study will reveal these two genera to have the same number of chromosomes: reported differences may be real, or they may result from difficulty in interpreting the cytological preparations.

SUMMARY

Chaptalia is an American genus of the Compositae. Four collections of herbarium material were made in Brazil for each of two species: *C. nutans* and *C. integrifolia.*

Chromosomes of these two species were studied: $2n = 48$; centromeres are median, submedian, or, in some of the smaller chromosomes, subterminal; 8 long chromosomes are present in both species, with 4 of them having median centromeres and 4 submedian.

Chaptalia is extremely difficult cytologically Burkart (1944) was unable to get definite counts of chromosomes for the two species investigated by him. Modification of Meyer's (1945) paradichlorobenzene technique gives excellent preparations for this genus.

C. nutans is the only weed in the genus: that condition is not correlated with differences in chromosome number.

Affinity of *Chaptalia* and *Gerbera* will likely be evidenced by cytological data.

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ANTIBIOTIC ACTIVITY OF CASSIA RETICULATA WILLD.[1]

WILLIAM J. ROBBINS, FREDERICK KAVANAGH AND J. D. THAYER

In the early fall of 1945 Dr. C. W. Dodge, Mycologist at the Missouri Botanical Garden, reported to one of us (W.J.R.) that an extract of the leaves of *Cassia reticulata* Willd. was taken by mouth by residents of Costa Rica as a cure for chronic gonorrhea. He generously supplied us with a small collection of the dried leaves and his record of the use of the plant.

According to his notes made in 1936, the plant is known locally as Sara-gundín or Mayadín. The directions call for placing 10 to 15 leaves in one pint of water, heating 10 to 20 minutes, cooling and taking in two or three doses; morning and evening if two and morning, noon and evening if three. The decoction is said to be good for one day only as it spoils easily. It is reported also to be unstable in alcohol.

Preliminary experiments were carried out with the leaves supplied by Dr. Dodge. Additional dried leaves were obtained from Guatemala through the courtesy of Mr. Ralph Pinkus and the identity of the plant was confirmed. No significant difference in the activity of the extract of the leaves recently collected and those collected 9 years ago was noted.

An extract prepared by boiling 5 g. of dried leaves with 50 ml. of distilled water was tested in beef broth on *Staphylococcus aureus*. The extract of 3 mg. of dried leaves in 1 ml. of medium completely inhibited growth. The aqueous extract was concentrated 5 times by boiling without reducing its activity. Almost all of the active material was removed from the aqueous extract of the leaves by repeated shaking with methyl-iso-butyl-ketone. The ketone extract retained its activity after drying at 100° C.

Isolation of an Active Crystalline Material. A yellow crystalline material with antibiotic activity was isolated from the *Cassia* leaves.

One hundred and fifty grams of dried leaves were boiled in 14 l. of water for an hour and the water decanted. The leaves were then boiled with 4 l. of water containing 5 g. sodium carbonate. The cooled extracts were combined, acidified to pH 3.2 with dilute hydrochloric acid and extracted with 500 ml. methyl-iso-butyl-ketone. The ketone was removed, and the extraction repeated with 300 ml. of the ketone. The ketone extracts were combined and subjected to distillation under reduced pressure. A total of 3.7 g. of material, including an oil with a characteristic odor and brown-colored solids, was obtained from the ketone extract.

This material was dissolved in acetone and most of it passed into ethyl ether by diluting the acetone with water. The ketone extract and the material soluble in ether inhibited *Staph. aureus* at a concentration of 60 μg. per ml. The ether was extracted six times with 1 per cent sodium bicarbonate solution. It was then extracted three times with 2 per cent sodium carbonate solution. The substances remaining in the ether after the sodium carbonate extractions were inactive on *Staph. aureus*. The substances in the sodium bicarbonate and sodium carbonate extracts obtained by acidification and extraction with ether inhibited *Staph. aureus* at a concentration of 32 μg. per ml. and had only slight effect on *Esch. coli* and *Kleb. pneumoniae*.

From the bicarbonate fraction an amorphous brick-red powder was isolated which inhibited *Staph. aureus* at a concentration of 8 μg. per ml. On slowly acidifying a dilute alkaline solution of the red powder, very fine, yellow, needle-like crystals formed. This crystalline substance was named cassic acid.

Cassic acid is not very soluble in alcohol, acetone, ether, chloroform, or methyl-iso-butyl-ketone; is slightly soluble in water and nearly insoluble in petroleum ether. It is a weakly acidic substance which crystallizes when a

TABLE 1. *Antibacterial activity of extracts of leaves of Cassia reticulata in μg. of dry material per ml.*

Observations made after 24 hrs. incubation. See text for preparation of extracts and fractions.

	Ketone extract	Bicar-bonate fraction	Carbonate fraction	Sodium salt of cassic acid
B. mycoides ATCC 9634	> 64	32	64	4
B. subtilis ATCC 9633	125	32	32	8
Staph. aureus ATCC 9144	64	64	32	8
Esch. coli ATCC 9637	> 1,000	> 1,000	> 1,000	> 250
Kleb. pneumoniae ATCC 9997	1,000	1,000	1,000	> 250
Pseudomonas aeruginosa ATCC 10145	> 1,000	> 1,000	1,000	> 250
Myco. phlei ATCC 10142	64	64	32	8
Myco. smegmatis (*smegma*) ATCC 10143	> 64	4	4	32

solution of the sodium salt is acidified to pH 5.5. The alkaline solution of cassic acid is of a reddish-pink color which changes through amber to yellow, as the solution is acidified. None of the elements sulphur, nitrogen, chlorine, bromine, or iodine was found by a qualitative analysis of crystaline cassic acid. There was no sharp melting-point. Melting and decomposition began about 330° C. and a small amount sublimed to form a red ring near the top of the capillary tube. An absorption spectrum of cassic acid determined by Mr. Morris Soodak of Fordham University showed peaks at 2700 and 4250 Å.

Bacterial Spectrum. The effects of the materials obtained by extraction with methyl-iso-butyl-ketone, the bicarbonate and carbonate fractions, and of cassic acid, all prepared as described in the preceding section, were determined by the dilution method for a number of bacteria (table I). All but the Mycobacteria were grown in beef broth; the Mycobacteria were cultivated in modified Kirschner solution. *Esch. coli, Kleb. pneumoniae,* and *Pseudomonas aeruginosa* were little affected by the antibiotic substances extracted from dried leaves of *Cassia reticulata. B. mycoides, B. subtilis, Staph. aureus, Myco. phlei,* and *Myco. smegmatis (smegma)* were all susceptible. Cassic acid was more active than the ketone extract or the carbonate fractions except for *Myco. smegmatis (smegma).*

Activity on Neisseria gonorrhea. Tests were made on two strains of *N. gonorrhea* in beef heart infusion broth enriched with 20 per cent ascitic fluid. Tests were incubated at 35–36° C in a 10 per cent carbon-dioxide atmosphere for 24 hours. The results showed *N. gonorrhea* and *Staph. aureus* to have a sensitivity to our preparations from *Cassia* of the same order of magnitude.

Fifty grams of dried leaves were extracted three times with 1 l. of boiling water. The extracts were combined and shaken out three times with 0.1 volume of methyl-iso-butyl-ketone and the ketone evaporated. The solids obtained (1.33 g.) inhibited *Staph. aureus* at a concentration of 64 μg. per ml. and *N. gonorrhea* at 84 μg. per ml. The solids from the ketone extract were dissolved in alcohol and petroleum ether added. The precipitate was removed, the alcoholic-petroleum ether solution was washed with water until all yellow color was removed from the petroleum ether. The aqueous washings were extracted with ethyl ether. The ether was removed, evaporated, the residue dissolved in acetone, and ten volumes of petroleum ether were added to the acetone. The precipitate which formed was centrifuged off and the solution evaporated to half volume. The precipitate which formed was removed and the solution taken to dryness. The solids amounted to 94 mg. and inhibited *Staph. aureus* at a concentration of 32 μg per ml. The solids were dissolved in ether and the ether extracted with 1 per cent NaHCO₃. The NaHCO₃ solution was acidified and extracted with ether which yielded 58 mg. of solids with an activity on *Staph. aureus* of 8 μg. per ml. and on *N. gonorrhea* of 4 μg. per ml. The ether after extraction with 1 per cent NaHCO₃ was extracted with 1 per cent Na₂CO₃. The Na₂CO₃ solution on acidification and extraction with ether gave 17.8 mg. of solids with an activity on *Staph. aureus* and on *N. gonorrhea* of 16 μg. per ml. The residual ether contained 4.7 mg. of solids which inhibited *Staph. aureus* at 64 μg. per ml. and *N. gonorrhea* at 32 μg. per ml.

A second lot of leaves was extracted with hot water and the extract shaken out with methyl-iso-butyl-ketone. The solids remaining after evapo-

ration of the ketone inhibited *Staph. aureus* at 62 µg. per ml. and *N. gonorrhea* at 84 µg. per ml.

The sodium salt of cassic acid inhibited *Staph. aureus* and *N. gonorrhea* at 8 µg. per ml.

Multiplicity of Antibacterial Substances from Cassia Leaves. Cassic acid is probably not the only antibacterial substance in the crude extract from the leaves of *C. reticulata*. This follows from the observation that the Na_2CO_3 fractions prepared as previously described had a far greater antibiotic activity for *Staph. aureus* than could be accounted for by the cassic acid content as judged from the red color developed in alkaline solution.

Animal Experiments. Preliminary experiments on a few animals were carried out. These indicated a low acute toxicity for mice and rabbits of preparations of *Cassia reticulata* when given by mouth and some excretion of material active against *Staph. aureus* in the urine.

Several mice were each given a total of 21 5 mg. of material (obtained by methyl-iso-butyl-ketone from a hot-water extract of the *Cassia* leaves) by means of a stomach tube (0.5 ml. at 10:00 a.m. and 0.5 ml. at 12:00 noon). The 21.5 mg. were obtained from about 800 mg. of dry leaves and had a total activity of 320 dilution units for *Staph. aureus*. Urine collected over a period of 10 hours was a dark reddish brown and showed activity on *Staph. aureus*.

By means of a stomach tube a rabbit was given 150 ml. of hot water extract of *Cassia* leaves at 9:25 a.m., at 11:00 a.m. and at 1:20 p.m. The total dosage of 450 ml. was equivalent to 22.5 g. of dry leaves and had an activity for *Staph. aureus* of 4,500 dilution units. The animal was catheterized and the urine removed before the start of the experiment. Subsequently, urine amounting to 236 ml. was collected over a period of 6 hours. By extracting the urine with methyl-iso-butyl-ketone a total activity of 800 dilution units for *Staph. aureus* was found.

A second rabbit received 3 grams of finely ground *Cassia* leaves suspended in 150 ml. of water at 9:50 a.m., at 11:00 a.m. and at 1:30 p.m., for a total of 9 g. Urine collected as before amounted to 130 ml. No activity for *Staph. aureus* was found in this urine.

A third rabbit received a concentrated water extract, 15 ml. at 10:00 a.m., 30 ml. at noon and 15 ml. at 2:00 p.m. The 60 ml. was the extract of 72.5 g. of dry leaves and its total activity was 14,500 dilution units for *Staph. aureus*. In 7½ hours after the third dose only 40 ml. of urine was obtained. The urine was tinged with blood and its total activity was 320 dilution units for *Staph. aureus*.

DISCUSSION

Species of *Cassia* (*C. fistula*, *C. marilandiea*, and others) have long been used as laxatives and some have been reported to be effective in other ways.

Watt and Breyer-Brandwijk[2] state that the natives of South Africa have used various species of *Cassia* for a variety of medicinal purposes. They record *Cassia petersiana* Bolle used for gonorrhea; a decoction of the root of *Cassia bearensis* Mig. for black water fever and schistosomiasis; a lotion of *Cassia sp.* for syphilis and yaws; *Cassia fistula* L. for malaria, black water fever, blood poisoning, anthrax, and dysentery; and other species for toothache, face eruptions, abdominal pain, body vermin, ulcers, or diarrhoea.

The efficacy of *Cassia reticulata* or preparations made from it in the treatment of gonorrhea was beyond the scope of our investigations. Although it is an interesting coincidence, to say the least, that this plant should be found to contain materials actively antibiotic for *N. gonorrhea*, a consideration of its use as described by Dr. Dodge would suggest that its therapeutic effectiveness is dubious.

According to Dr. Dodge, from 10 to 15 leaves are boiled in 1 pint of water. This would be equivalent to 2 or 3 g. of dried leaves in 500 ml. of water or the extract of 4 to 6 mg. in 1 ml. Our results indicate that such an extract would completely inhibit *Staph. aureus* or *N. gonorrhea*. However, it seems unlikely that the antibiotic material contained in the tea would be effective in vivo. Even if complete absorption of the antibiotic material in a single dose (150–250 ml.) occurred with no destruction, the blood of an adult would contain the equivalent of less than 0.3 mg. of dried leaves per ml. which is below the antibiotic level in vitro in our experiments.

There is, of course, the possibility that the antibiotic materials in fresh leaves are more active than those in the dried leaves which were the material for our experiments. This would perhaps be suggested by the report—if correct—that the active material from the fresh leaves is unstable in alcohol. The substances with which we worked were not noticeably affected by alcohol. It is possible also that the activity might be greater in vivo than in vitro or might be concentrated in the affected parts. In any event, further investigation of *Cassia reticulata*, its antibiotic properties and reputed therapeutic activity, would seem desirable.

SUMMARY

A tea made from leaves of *Cassia reticulata* has been reputed to be used in Central America in the treatment for chronic gonorrhea. A hot water extract of 2–5 mg. of dried leaves of *Cassia reticulata* in 1 ml. of beef broth was found to inhibit *Staph. aureus*. Most of the active material was removed from the water extract by methyl-iso-butyl-ketone. The material in the ketone extract inhibited *Staph. aureus* and *Myco. phlei* at a concentration of 64 μg. per ml. of nutrient medium, *N. gonorrhea* at 84 μg. per ml., *B. subtilis* at 125 μg. and *Myco. smegmatis (smegma)* and *B. mycoides* at more than

2 **John M. Watt & Maria G. Breyer-Brandwijk.** The medicinal and poisonous plants of southern Africa. E. and S. Livingston. Edinburgh. 314 pp. 1932.

64 μg. per ml. It was inactive for *Esch. coli, Kleb. pneumoniae* and *Pseud. aeruginosa.* A yellowish crystalline substance named cassic acid was isolated. Cassic acid was not active on *Esch. coli, Kleb. pneumoniae* and *Pseud. aeruginosa. B. mycoides, B. subtilis, Staph. aureus, Myco. phlei* and *N. gonorrhœa* were completely inhibited at concentrations of 4 to 8 μg. of cassic acid per ml. of medium. *Myco. smegmatis (smegma)* was inhibited by 32 μg. per ml.

Evidence was obtained that cassic acid is not the only antibiotic material in the extracts from dried *Cassia* leaves. The hot-water extract and the ketone extract of *Cassia* leaves when given by mouth were not acutely toxic to mice or rabbits though injury was observed with large doses. Some active material was excreted in the urine.

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MORPHOLOGICAL AND PHYSIOLOGICAL FACTORS IN STREPTOMYCIN PRODUCTION

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The production of streptomycin has been intensively investigated since its discovery by Schatz, Waksman, and Bugie in 1944 (9). Most of the biological research efforts following its discovery have been directed to the production of high-yielding strains of the organism and the formulation of media in which the synthesis of streptomycin is increased. Very little published information is available, however, on the nature of the fermentation, except for a paper by Waksman *et al.* (11). The investigations reported in this paper deal with some morphological and physiological changes occurring in the culture medium during the growth of *Streptomyces griseus* and production of streptomycin.

General Methods. The organisms used in these studies were single-colony isolates from a culture of *Streptomyces griseus*, which had originally been obtained from Dr. S. A. Waksman as his strain nine. Three different media were used for most of these experiments, only one of which (C) was chemically defined. These were:

Medium A	Medium B	Medium C[1]
Cerelose[2]—10 gm.	Cerelose—5 gm.	Cerelose—10 gm.
Bacto peptone—5 gm	Bacto peptone—6 gm.	KH_2PO_4—2.38 gm.
Sodium chloride—5 gm.	Beef extract—5 gm.	$K_2HPO_4 \cdot 3H_2O$—5.65 gm.
Corn steep liquor—15 gm.	Bacto yeast—3 gm.	Na lactate—11.2 gm.
Distilled water—1000 ml.	Distilled water—1000 ml.	$(NH_4)_2SO_4$—2.64 gm.
		$MgCl_2$—1.21 gm.
		$Zn SO_4 \cdot 7H_2O$—0.0115 gm.
		$Fe SO_4 \cdot 7H_2O$—0.0111 gm.
		$Cu SO_4$—0.0064 gm.
		$Mn Cl_2 \cdot 4H_2O$—0.0079 gm.

The pH of the media was adjusted to 6.95–7.0 before sterilization in most experiments, and the media were autoclaved at 15 pounds for 20 minutes. One hundred and twenty-five milliliters of media were used in each 500-ml. Erlenmeyer flask. The media were agitated and aërated in a reciprocal shaker at 107 strokes per minute with a stroke length of 7 cm. Mycelial "seed" was obtained by adding 5 ml. of water to a five-day-old culture of the fungus, rubbing the surface of the culture with a loop, and transferring the spore

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[1] Medium C is a modification of the synthetic medium formulated by Dr. H. H. Thornberry, and its publication is in press.

[2] Cerelose is a grade of commercial dextrose.

suspension to a flask containing the medium. After 24–48 hours' growth on the shaker, about 2 ml. of this mycelial suspension were added to each flask of the experimental media. The fungus was grown at 26° C for at least 216 hours in most experiments and samples were assayed at various intervals of time according to the method described by Loo *et al.* (7). All experiments were run in triplicate and the results reported in this paper are always averages of the data from three flasks. Determinations of growth, viscosity, streptomycin production, and hydrogen-ion concentration were made from same samples.

When investigating biologic materials, the amount of variation among replicate samples is always an important factor and averages of triplicate flasks do not always represent a true picture. Nevertheless if the averages obtained from such triplicate samples tend to fall consistently along curves which greatly resemble each other, it is reasonable to assume that a fairly accurate picture of the changes occurring in the media is presented by such curves. The data in these investigations are therefore presented graphically rather than in the form of tables.

Growth. The growth of *S. griscus* in "shake" culture can be initiated from either spores or mycelium. Germination of spores was difficult to observe even under magnification obtained with an oil-immersion objective and appeared only as an elongation of the spore. Studies by Carvajal with the electron microscope, however, revealed that a germ tube is produced (1). When mycelial seed is used the hyphal segments, produced by the agitation of the mycelium, are the source for the growth in culture. Shortly after the hyphae began to elongate a definite incipient rounding up of the mycelium was visible. Weft formation was always evident after six hours. The number of wefts increased during the next six-hour period, and as they matured formed round or elongated mycelium clumps. Occasionally the mycelium formed more pellet-like structures which were readily visible to the naked eye and resembled the pellets produced during the penicillin fermentation. These mycelial clumps consisted of a dark, dense center resembling microsclerotia and a peripheral zone of intermingling, branched, growing hyphae which radiated from the central core. By 24–30 hours their number had increased and the peripheral hyphae had so intermingled with those of neighboring balls as to form a network of hyphae throughout the entire medium. No further changes were evident until about the 54–60-hour period. A breaking up of the mycelium then occurred; the clumps were more distinctly separated and short chains of spores were observed. This disintegrating process continued, and between 72 and 84 hours further fragmentation of the peripheral hyphae and central core occurred so that the ball structure disappeared. Short mycelial pieces and dense granular fragments were dispersed through the entire medium. Spore formation evidently had been accelerated since the

medium contained a great number of spores, either single or in chains of from two to four. No further changes of the culture were evident, though observations were continued until the cultures were 240 hours old. Growth phases were studied in three different media, and the same general pattern of growth was observed. Nevertheless the growth of *S. griseus* in the chemically defined medium, C, showed some differences. The fragmentation of mycelium was less evident, and hyphal segments were not so common in the later stages of growth. Almost the entire fungus material in later stages consisted of spores.

A study of mycelial growth was also made by gravimetric methods. Three flasks were taken at random from the shaker at various intervals of time and the entire cultures centrifuged in weighed tubes. After decanting the supernatant medium, the mycelium and tube were dried at 80° C for 36 hours, and the mass of mycelium obtained by the difference in weight. No marked gain in mass occurred during the first six hours, after which the mycelial weight slowly rose until about the twelve-hour period. It then increased rapidly and the amount of hyphae reached a maximum between 30 and 36 hours. The weight rapidly decreased, between 60 and 70 per cent, during the 24 hours after the peak had been reached. Generally the decrease tended to be slower after 72–96 hours. Typical growth curves are shown in figure 1. The growth phase of the curve was similar for all media, but the decline phase showed some variation. In the synthetic medium, C, the decrease from maximum weight was only 12 per cent for the first 24 hours following the peak and had only decreased 20 per cent even after 132 hours. It is apparent that the centrifuging and drying procedure for obtaining mycelial weight has some possibility of inherent inaccuracy because other solids in the media would be weighed at the same time. Microscopic examination, however, showed very little insoluble material to be present in the medium during the growth of the micro-organism. Any such deviations would be small compared to the weight of the entire mycelium.

One can postulate from the disintegration and from the decrease in the mass of mycelium that lysis of the hyphae occurred during the decline phase of the culture. The observation that the total mass of material in medium C, where spore formation was abundant in the later stages, showed only small decreases can indicate that the spores were more resistant to lysis than the mycelium.

Changes in the viscosity of the culture have been noticed during the growth of *S. griseus* in shake culture. An increased viscosity during the growth phase occurred in all media. This change has been attributed to the production of gums and to the admixture of air to the medium during shaking. The phenomenon was investigated by measuring the apparent viscosity of the same samples which were used for weight measurements in an Ostwald viscometer. The increase of viscosity closely followed that of my-

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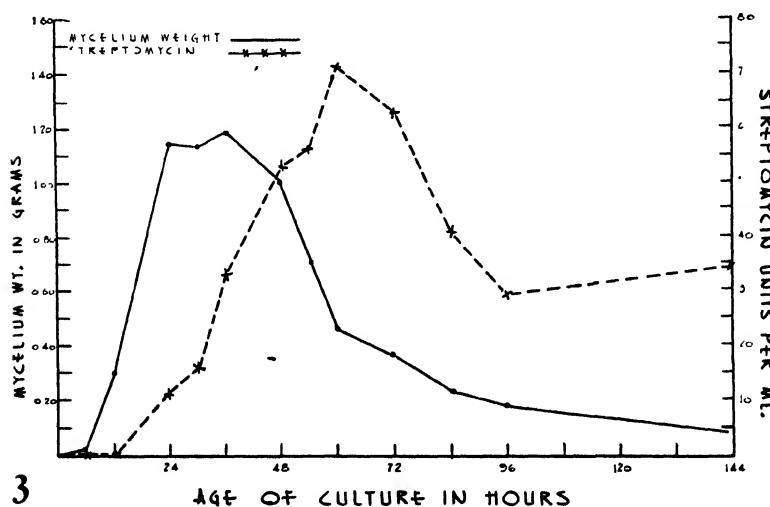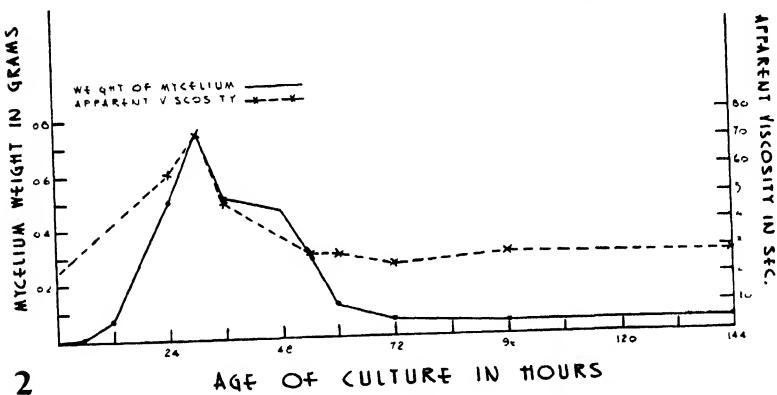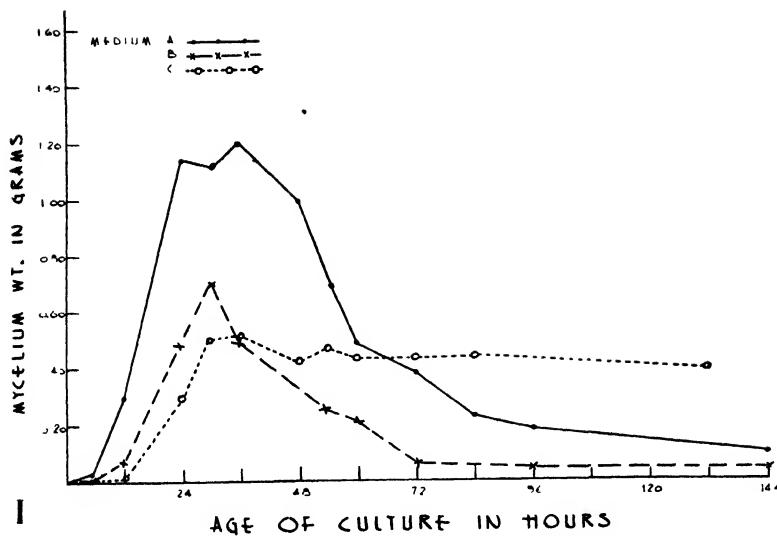
celium production and reached a maximum level at the same period as mycelial weight in all experiments (fig. 2). A decrease in viscosity then took place. The supernatant liquid obtained after centrifuging the culture showed no significant increase over non-inoculated media. The viscosity of shake cultures is thus closely related to the amount of interwoven mycelium in the culture.

Production of Streptomycin. The production of streptomycin during the growth of *S. griseus* is dependent on many factors. Among these are the constitution of the medium, hydrogen-ion concentration, temperature, oxygen supply, agitation of the medium, and others.

When the culture is grown under conditions previously described, most of these factors are adequate, though perhaps not optimum for the production of streptomycin. The production of streptomycin followed the same general pattern in all the different media (fig. 3). No streptomycin was detected in the solution during the first 12 hours. The amount increased steadily from then on until a maximum was reached between 48 and 60 hours of growth. A decline in the streptomycin content of the solution occurred after this time. This decrease continued until after about 96 hours when the slope of the curve decreased and a tendency to level off was evident. In all cases the peak of the streptomycin curve lagged behind the peak of the growth curve by about 24 hours (fig. 3). Streptomycin production in the medium therefore is not primarily a function of the active growth phase of *S. griseus*. Only about 25 to 50 per cent of the total streptomycin had been released in the solution by the time the growth peak was reached.

Hydrogen-Ion Concentration. All media were adjusted to pH 6.5–6.75 before seeding. Neither autoclaving of the medium nor the addition of the "seed" materially affected the pH. Determinations of hydrogen-ion concentration were made with a Beckmann glass electrode on the same samples which were used for growth and streptomycin production.

The pH changes could be divided into two stages: the first occurred during the period of active growth and the second during the later stages (fig. 4). The variation of pH in the first period was dependent upon the medium used. When grown in Medium A, which contained corn-steep liquor, an increase occurred during the first 12 hours, followed by a decrease until about 36 hours. A similar medium, B, containing beef extract and Bacto yeast in place of the corn-steep liquor showed a gradual increase during the same time. The pH values in a synthetic medium, C, decreased slightly during the first 36 hours and showed no initial rise. The pH of all media invariably rose during

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Explanation of figures 1–3

FIG. 1. Growth of *Streptomyces griseus* in shake culture.   FIG. 2. Relation between growth and viscosity in a corn steep medium.   FIG. 3. Growth and streptomycin production in a corn steep medium.

the second phase and the slope of the curve began to level off at about 96 hours, at which time the values were around pH 8.5.

No correlation could be made between the ability of a strain of *S. griseus* to produce streptomycin and the pH changes in the culture medium. Four
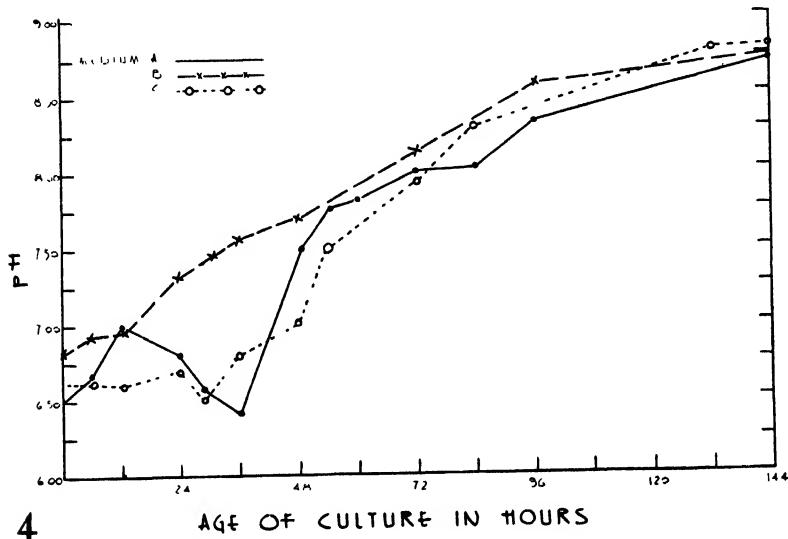
4   AGE OF CULTURE IN HOURS
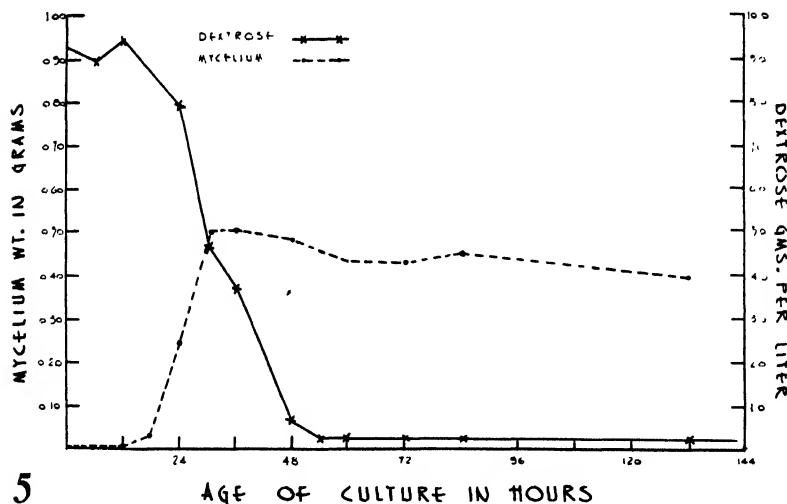
5   AGE OF CULTURE IN HOURS

Fig. 4. Changes in hydrogen-ion concentration. Fig. 5. Growth and dextrose consumption in a synthetic medium.

different strains of *S. griseus* which produced 360, 365, 205, and 215 units of streptomycin per ml. all had about the same pH curves on the corn-steep liquor medium. Other strains which have been obtained by ultra violet irradi-

ation and produced no measurable amount of streptomycin, caused a rise in pH to 7.85 at the end of 48 hours, while the parent strain which produced 240 μ/ml. changed the pH to 7.65.

Aerobic Nature of Streptomycin Production.   That the biosynthesis of streptomycin is dependent upon the amount of the air supplied to a medium is known and oxygen is assumed to be the essential component. Good aëration is usually supplied either by agitation of the medium or, in commercial practice, by forcing air through the medium. The importance of air for the growth of this Actinomycete was readily demonstrated since *S. griseus* did not grow to any extent when surface cultures were seeded with the fungus and the contents of the flasks then washed with filtered tank nitrogen. The reaction responsible for the production of streptomycin, however, might have a different oxygen relationship, since streptomycin formation appeared to be a function of the mature mycelium and was not intimately associated with the process of growth. After the growth phase was completed some anaërobic mechanism might be responsible for streptomycin production. A series of shake culture experiments were set to test this possibility. The flasks were seeded in the usual manner. Some flasks were treated by passing filtered nitrogen through the medium for 15 minutes while the flasks were shaken to remove any free oxygen. These flasks were then immediately sealed. Other flasks were not washed with nitrogen until 30 hours had elapsed and maximum growth reached. The remainder were allowed to grow under the usual conditions of aëration by agitation. The data recorded in table 1 showed that

TABLE 1. *Oxygen and synthesis of streptomycin.*

Treatment	Growth	Units of streptomycin μ/ml.	Average
No nitrogen added	Good	240 240 240	240
Nitrogen added at 0 hrs.	V. slt.	0 0 0	0
Nitrogen added at 30 hrs.	Good	16 16 16	16

no streptomycin was produced if air was excluded, at the start, though some mycelial growth occurred. When air was excluded after growth had nearly reached a maximum at 30 hours, only the small amount of streptomycin produced up to that time was found even at the 72-hour assay. Both good streptomycin production and good growth was obtained when no nitrogen was used. The synthesis of streptomycin therefore is dependent upon the availability of air to the fungus.

Carbohydrate Consumption.   Studies of dextrose consumption during the growth of *S. griseus* in "shake flasks" can best be made when the organism is grown in a synthetic medium. Actinomycetes grown on more complex media containing peptone or beef extracts have been shown to utilize such materials as a source of carbon under certain conditions (5). Other fungi have also been shown to assimilate amino acids to satisfy their carbon requirements (2). Thus a more accurate picture of the assimilation of dextrose can be obtained when no other possible source of carbon is present in the medium. The procedure used in the analysis of dextrose was that described by Lathrop and Holmes (6) as modified by Lott (8).

Very little change occurred in the dextrose content of the medium during the first twelve hours of growth. This is the period when mycelial weight measurements also showed very small weight increases. Between 12 and 48 hours the sugar was almost entirely consumed (fig. 5). The dextrose in the medium dropped from 9.6 grams per liter to 0.75 grams per liter. The curve leveled off at 54 hours when the dextrose reached 0.1 per cent of the original concentration. At the time when the weight of mycelium reached its peak, 36 hours, 61 per cent of the initial dextrose had been assimilated and the remainder was rapidly utilized during the next 18 hours, a period when no new mycelium was produced.

Consumption of Minerals.   A study of the total changes in the mineral content of the synthetic medium during growth was made by conductometric measurements. The specific conductivity of the medium decrease slowly but steadily during the first 36 hours, from 2450 to 2100 reciprocal ohms. An increase in conductivity occurred at 48 hours, but at 54 hours the values again were about 2100 and remained at that level for the rest of the run. Unless other readily ionizable materials were produced during this period, the total amount of mineral in the medium was not greatly depleted during mycelium formation.

DISCUSSION

The data accumulated in this study indicate that certain correlations exist in shake culture irrespective of the medium used. Viscosity changes in the medium seem dependent upon the growth of the mycelium. With increasing weight an increase in the viscosity occurs, and the peaks for mycelial weight and viscosity are simultaneous. Both peaks are then followed by a decrease in their respective values. This phenomenon was also verified by microscopic observations, which showed an increased amount of growth and intermingling of the hyphae until about the time at which the maximum weight occurred. The profuse and general intermingling of the hyphae throughout the medium could thus reduce the free movement of the liquid portion of the culture and bring about an increased viscosity. Because both

the weight and viscosity measurements decrease before any apparent frag-
mentation of the hyphae is visible the decline of the culture most probably
begins soon after maximum growth has occurred.

The production of streptomycin is not dependent solely upon the growth
of *S. griseus*. Many other factors such as high temperatures, which allow opti-
mum growth inhibit streptomycin synthesis (10). Similarly, on surface cul-
tures some carbohydrates will furnish material for good growth, but poor
streptomycin production (3). Under favorable conditions, however, strepto-
mycin production is dependent upon the amount of mycelium produced. The
period of maximum accumulation of streptomycin in the medium, however,
invariably lagged behind that period of maximum growth. These results are
at variance with those recently reported for surface culture in which "the
maximum concentration of streptomycin corresponded to the peak of growth
for the organism" (11). Two explanations of these data are evident, (1) that
the synthesis of streptomycin can take place in a "declining" mycelium, and
(2) that because of the degradation of the mycelium the streptomycin previ-
ously synthesized is more readily released. Collateral evidence for the second
explanation is that streptomycin is always found in the mycelium of culture
at the time the solution contains its maximum quantity. Sometimes this
residual streptomycin is as high as 20 per cent. As noted by Waksman *et al.*
(11), the streptomycin content of the medium decreases after the peak has
been reached and this decrease usually occurs about the time fragmentation
of the hyphae is observed. The cause of the gradual reduction in the strepto-
mycin content of the medium is still a moot question.

Studies of the changes in hydrogen-ion concentration during the produc-
tion indicate that such changes can be divided into two phases. The first stage
is one of active growth and usually is completed in 36 hours. During this
stage the pH changes are dependent upon the nature of the medium. An
initial increase occurs in some media, which is followed by a decrease. In
other media an increasing pH is evident from the start. The second stage is
similar in all media; it is a period of increasing alkalinity with a tendency
to level off after 96 hours. The increased pH begins after the maximum
growth has been reached and decrease in mycelial weight has begun. Such
a high pH during the "decline" of the culture might be due to the liberation
of ammonia and basic organic nitrogen compounds during the degradation
of the mycelium (4).

SUMMARY

1. Microscopic observations and mycelial weight measurements indicated
that the growth of *S. griseus* in shake culture reached a maximum, after
which the culture declined and lysis and fragmentation of the mycelium
occurred.

2. The viscosity of the culture was directly related to growth.

3. The peak of streptomycin production lagged behind the growth peak.

4. Changes in pH can be divided into two phases, those occurring during growth, and those associated with the "decline" of the culture.

5. The ability of various strains of *S. griseus* to raise the pH of the medium was not associated with their ability to produce streptomycin.

6. The synthesis of streptomycin proceeded only in the presence of oxygen.

7. Very little change occurred in the specific conductivity of the medium during fermentation.

8. The synthetic medium was depleted of 60 per cent of the original dextrose content before the peak for streptomycin production was usually reached.

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ESTIMATION OF ANTIBACTERIAL SUBSTANCES BY SERIAL DILUTION METHODS

FREDERICK KAVANAGH

Incomplete search through the plant kingdom for antibacterial substances has revealed more than fifty named agents that are active in suppressing the growth of suitable test organisms. Some of these substances have been so poorly characterized that the same one may appear more than once in a complete list of antibacterial substances of natural origin because it was obtained from two or more different plants. The discovery of each new antibacterial substance increases the difficulty of distinguishing which are new and which are only old substances from new sources, and may create new problems in quantitative estimation.

Ideally, the methods of assay and identification of antibacterial substances would be specific chemical methods which could be applied to dilute solutions containing many other substances. When more is learned about the chemistry of each antibacterial substance, probably such specific chemical methods for their identification and determination may be devised. Until this is done, antibacterial tests will have to be used both to characterize the substance and to follow it during the process of isolation and purification. Though the assay methods employ relatively few species, the tests made for purposes of identification require the use of many bacteria. The details of the tests used in identification, however, are the same as those used in the assay methods and need not be described separately.

In the production, concentration, and isolation of an antibacterial substance, the most frequently repeated operation is the determination of the quantity of active substance. This is done by measuring the inhibitory action of the substance on the growth of a test organism. Three general methods of assay, each of which has several modifications, have been used. These are: the streak-plate; the cup-plate; and the serial dilution method. Each method has certain advantages and disadvantages, some of which will be mentioned.

In the streak-plate method (5, 19), the antibacterial substance is diluted in nutrient agar, the agar poured into petri dishes, and the surface of the agar streaked with bacteria. After a period of incubation, the highest dilution of the antibacterial substance that inhibits the growth of bacteria on the surface of the agar is chosen as the end-point and the activity is computed from this dilution. Several species of bacteria can be streaked on the same plate. The dilution steps are usually rather large and the activities so ob-

tained have a large systematic uncertainty. The method is useful in various surveys, however, because the approximate activity against several organisms can be measured with a relatively small expenditure of material and labor.

In the cup-plate method (5, 10, 16, 18) short cylinders are placed on the surface of agar that has been inoculated with a suitable test organism; some of these cups are filled with solutions of known concentrations of a reference substance, preferably the one being assayed; the remainder of the cups are filled with dilutions of the sample of unknown concentration; the plates are incubated for about eighteen hours; and the zone of inhibition around each cup is measured to the nearest 0.5 mm. The diameter of the zone of inhibition is directly proportional to the logarithm of the concentration of the anti-bacterial substance for a certain range of concentrations. To achieve the precision of which the method is capable, very exact control of the many factors that influence the diameter of the zone of inhibition must be maintained. A laboratory which does few tests is not likely to have either the trained personnel or the equipment necessary for attaining high accuracy. One of the advantages claimed for the cup-plate test is that the sample assayed need not be sterile. This would seem to be a dubious advantage in determining penicillin, which is so susceptible to the penicillinase produced by many bacteria. For assaying other substances, which are not affected by bacterial enzymes, however, the use of a non-sterile sample is advantageous, especially with thermolabile substances which are adsorbed by Seitz filters. The cup-plate method is also used in testing the action of substances against fungus spores suspended in the agar. The paper-disc (11, 17) method is a variant of the cup-plate method.

In the serial dilution method (5, 7, 12) the dilutions of the antibacterial substances are made in tubes of nutrient broth. The method involves the following steps: (1) dilution of the active substance in nutrient broth; (2) inoculation of the serial dilutions with the test organism; (3) estimation of an endpoint after a period of incubation; and (4) computation of the activity of the substance from the dilution of the endpoint. The materials required are a sterile sample of the substance to be tested, sterile test-tubes and pipettes, and a clear medium in which the test organism will grow. Five advantages of the dilution tube method are: (1) the technique is simple and easily learned; (2) the factors influencing the test are few or can be controlled for long periods of time; (3) the test is capable of fairly high precision; (4) the activity of an active substance can be expressed as a number which is directly proportional to its concentration (this is also true of the streak-plate method); (5) a number of useful modifications are possible.

Theoretically, the concentration determined by the cup-plate method is the same as that put into the cup, whereas the concentration determined by the dilution method lies between two values, neither of which is necessarily

the same as that of the sample, although one of them is arbitrarily taken to be its concentration. The uncertainty thus introduced will depend upon the type of test used and is less than 20 per cent for the arithmetic-series dilution method, or about the same as the error of the cup-plate method as it is usually performed. In fact, some laboratories (15) have found a dilution method to be twice as precise as the cup-plate method; their error for the dilution method was about 10 per cent. Which method is to be used in an investigation will usually be determined by considerations other than those of accuracy and precision.

Several quick dilution tests that have special and limited application are described in the literature. In one test (8) very large numbers of bacteria were added to tubes containing the antibacterial substance, the tubes were incubated for 1.5–4 hr., and the turbidity was measured; the greater the amount of the active substance the less was the turbidity. In the nitrite method (6) for estimating penicillin, an incubation period of from 1 to 1.5 hours was sufficient.

In another test (13) a strain of hemolytic streptococcus was used, and after a short period of incubation, 0.5–1 hour, in the presence of red blood cells in a broth medium, the highest dilution showing no trace of hemolysis was taken as the endpoint.

Sometimes the selection of the endpoint in the dilution test is not certain, especially when part of the population of the test bacteria are resistant to the active substance. In the streak and cup-plate methods, the resistant organisms form isolated colonies in the zone of inhibition and can be ignored in evaluating the test. In the dilution tube method, these organisms cause turbidity where only clear tubes should be and make uncertain the interpretations of the test. Therefore, strains of bacteria[1] having few resistant organisms should be used for dilution tests. The serial dilution methods to be described have been used for four years and have been found to be adequate for most identifications and measurements.

The dilution tube method and its modifications, as used in this laboratory, are discussed under the following heads: (1) the bacteria, their selection and maintenance; (2) the test-media and physical condition for the tests; (3) the technic of the tests and the interpretation of assays; and (4) the possible errors introduced by mechanical imperfections and biological variations.

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[1] It is not certain whether the bacterial population contains members resistant to the antibacterial substance or whether the resistant members develop their resistance in the presence of it. For the sake of simplicity of statement, the assumption will be made that the population contained the resistant members before exposure to the active substance. Demerec (1) has given evidence that penicillin resistance in *Staphylococcus aureus* is the result of mutation.

THE BACTERIA

Selection of Bacteria. The strain of bacteria used depends upon the object of the test, which may be: the determination of the potency of culture liquids and the fractions obtained from them in the process of purification; the determination of the characteristics of the active substance by measuring its activity against standard strains of bacteria; the determination of the activity against bacteria resistant[2] to other antibacterial substances. Most tests are made to determine the potency of various preparations.

Standard Strains of Bacteria. The use of different strains and species of bacteria makes difficult the comparison of results obtained by different investigators, since strains of a species of bacteria may respond differently to an antibacterial substance. If the same strains of bacteria were used by all investigators for characterization, possible identity would be immediately obvious.

To establish a standard set of bacteria for our tests, certain of the standard tester strains of S. A. Waksman, supplemented by other species of bacteria which have been found useful, were selected. As many as possible of the standard tester strains of S. A. Waksman are used because the greatest number of substances have been tested against these strains. The bacteria selected are relatively non-pathogenic and are easily maintained. While these bacteria are sufficient for most purposes, it may be necessary to use many more species in studying the production and purification of an active substance.

The following three strains of Gram-positive bacteria seem to be all of this group that are necessary for characterizing an antibacterial substance: *Staphylococcus aureus*, Heatley strain (ATCC 9144), hereafter written *Staph. aureus* H., was obtained from G. W. Rake of the Squibb Institute for Medical Research; *Bacillus subtilis* (ATCC 9633), and *Bacillus mycoides* (ATCC 9634), are two of the standard tester strains of S. A. Waksman.

The Gram-negative bacteria should include the *Escherichia coli* (ATCC

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[2] The tests using resistant bacteria, while few, are important because they frequently establish whether the particular substance is a new one. The bacteria used for this purpose are derived from the Heatley strain of *Staph. aureus*. Since they are resistant to one or more known substances, they are useful in identifying such substances. Strains resistant to three substances have been developed. The resistant strains are used to establish lack of identity of two or more substances. Consider the following example. Penicillin and an unknown antibacterial substance are tested against a penicillin resistant strain of bacteria, and the bacteria are found to be susceptible to the unknown. One could say then, with certainty, that the unknown was not penicillin. If the bacteria had been resistant to penicillin and the unknown, one would know that either the two substances act the same or that resistance to the unknown had developed. It is this latter possibility which makes the results ambiguous when the bacteria resistant to a known substance are also resistant to the unidentified one. Antibacterial identity of two substances does not guarantee chemical identity, which can be established only by chemical means.

9637) that is the standard tester strain of S. A. Waksman, *Klebsiella pneumonia*[3] (ATCC 9997), and *Pseudomonas aeruginosa* (ATCC 10145).

In addition to the Gram-positive and Gram-negative bacteria, the Doudoroff strain of *Photobacterium Fischeri* is valuable for these tests.

Acid-fast bacteria are used in certain survey work. The following strains grow fairly rapidly in the modified Kirchner medium and are relatively non-pathogenic: *Mycobacterium smegmatis* (smegma) ATCC 10143, *Myco. lacticola, Myco. phlei* ATCC 10142, *Myco. avium, Myco. berolinensis,* and *Myco. ranae.*

For the determination of the potency of crude culture fluids, chemically fractionated preparations, and animal fluids usually only one of the following species of bacteria need be used: *Staph. aureus* H., *E. coli, B. subtilis,* and *Kleb. pneumoniae.* We use *Staph. aureus* H. for routine determination of the potency of all antibacterial substances formed by the Basidiomycetes.

Maintenance of Stock Cultures.   The media used in testing antibacterial substances fall into two groups, those used for propagation of the bacterial cultures and those used in the dilution tests. All of the 30 species and strains of bacteria, except the *Photobacterium,* maintained in this laboratory grow satisfactorily on the Bacto–A.C. medium used in the form of broth and agar slants. This medium contains per liter: 5 g. dextrose, 3 g. Difco malt extract, 3 g. Difco Bacto-Beef Extract, 3 g. Bacto-yeast extract, and 20 g. Proteose Peptone No. 3 Difco. The pH is 6.3 after sterilization. The medium for the agar slants is made by adding 15 g. agar to one liter of the A.C. broth. The *Mycobacteria* are maintained in a modified Kirchner medium in which they form a uniform suspension without a surface pellicle. The *Photobacterium* culture is maintained in an artificial sea-water broth or on the E-Y agar.

Weekly transfers of the bacteria (other than *Mycobacterium*) from agar slants to freshly sloped A.C. agar slants are made. The tubes are incubated at the appropriate temperature for 24 or 48 hours, depending upon the rate of growth, and are then stored at 11° C until needed. A complete set of cultures transferred at monthly intervals is kept in the refrigerator at 4° C.

The *Mycobacteria* are grown in the modified Kirchner medium and fresh transfers are made every three days. The tubes are kept at 36° C until transfers are made to fresh broth. The stock culture of *Photobacterium* can be maintained in tubes of the artificial sea-water solutions, in which they may remain viable for at least four months at 15° C. *B. subtilis* and *B. mycoides* are grown at 30° C, and all other bacteria are grown at 36–37° C.

The agar medium on which Egorova and Yarmolink (4) grew *Bact. Issatchenkoi* and kept its light-producing ability for twenty-three years is

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[3] This strain is useful for streptothricin and streptomycin assays because it contains very few individuals resistant to these substances.

much better for maintaining stock cultures of photobacteria than the sea-water broth solidified with agar. The formula for E.Y. agar, modified by the substitution of Bacto-peptone for Witte Peptone and water for the fish extract is: NaCl 30 g., K_2HPO_4 1 g., $MgSO_4 \cdot 7H_2O$ 0.5 g., asparagine 5 g., Bacto-peptone 10 g., agar 15 g., distilled water 1000 ml., pH after sterilization 6.6.

TEST-MEDIA AND PHYSICAL CONDITIONS FOR THE TESTS

In general, the test-media are not as rich as the A.C. medium and the growth of the bacteria is slower than it is in the A.C. medium. The test-media should not contain appreciable amounts of substances which inhibit the action of the antibacterial substances.

Four test-media are necessary when the bacteria mentioned previously are used. Assays in which *Staph. aureus* H., *E. coli*, *Kleb. pneumoniae*, and *Ps. aeruginosa* are used are performed in beef extract (B.E.) medium which contains 5 g. of Bacto-Peptone and 3 g. Bacto-Beef Extract per liter of solution. The pH after sterilization is 6.8. The B.E.D. medium is beef extract to which 5 g. dextrose is added to each liter of B.E. medium before sterilizing; it is used for assays with *B. subtilis* and *B. mycoides*. The pH is 6.6 after sterilization. The mycobacteria are maintained in the "growth medium" and tested in the "test medium." the composition of which is shown in table 1.

TABLE 1. *Composition of the two modified Kirchner's media for the Mycobacteria.*

	Growth-Medium	Test-Medium
Asparagine	5 g.	5 g.
Glycerol	20 g.	20 g.
Lecithin	0.5 g.	0.1 g.
Tween 80	0.5 g.	0.1 g.
Na_2HPO_4	3 g.	3 g.
KH_2PO_4	4 g.	4 g.
$MgSO_4 \cdot 7H_2O$	0.6 g.	0.6 g.
Na citrate	2.5 g.	2.5 g.
Fe NH_4 citrate	0.05 g.	0.05 g.
Water	1000 ml.	1000 ml.
pH		6.6

The Tween 80 is a wetting agent made by the Atlas Powder Co. Dubos (3) introduced the use of Tween 80 and lecithin in media for mycobacteria. The *Photobacterium* is tested in an artificial sea water (14) medium which has the following composition: 26.7 g. NaCl, 0.71 g. KCl, 1.15 g. $CaCl_2$, 6.81 g. $MgSO_4 \cdot 7H_2O$, 2 g. peptone, and distilled water 1000 ml. The pH after sterilization is 6.2–6.6. The inoculum is prepared by inoculating a flask of the artificial sea-water with several ml. of the stock *Photobacterium* culture or cells scraped from the surface of a culture growth on E.Y. agar, and incubating this at 15° C for 48 hours. A flask of inoculum is prepared because a large volume of inoculum is needed for the antiluminescent tests (9) which are usually done at the same time as the antibacterial tests described here.

Test Inoculum. The inoculum for most of the bacteria is prepared by transferring a large number of bacteria from an A.C. agar slant to a tube of A.C. broth and incubating it for 6 hours at 36°. *B. subtilis* and *B. mycoides,* however, are grown for 24 hours at 30° C. The one- or two-days-old cultures of the mycobacteria in the Kirchner's broth are used as the inoculum.

The 24-hour cultures of *B. subtilis* and *B. mycoides,* are diluted 1000 times in B.E.D. broth. The 24- or 48-hour cultures of mycobacteria are diluted 50 times in the modified Kirchner's test-medium. The 48-hours-old culture of the *Photobacterium Fischeri* is diluted 500-fold with artificial sea-water just before using. The six-hour A.C. broth cultures of the other bacteria are diluted one million times in the B.E. medium to give a concentration of bacteria of from 500 to 2000 per ml.

CONCENTRATION OF BACTERIA

The minimum inhibitory concentration of an antibacterial substance may increase greatly with an increase in the concentration of bacteria used in the test because of the concomitant increase in the number of bacteria resistant to it. When large inocula are used, the bacteria that actually function in the test are the few that are resistant and not the greater part of the population that is sensitive to the active substance. This is the reason that small inocula are used in the methods described here.

The minimum concentration of an antibacterial substance that inhibits a particular strain of bacteria is measured for concentrations of bacteria of about 1,000 cells per ml. Then the strain is tested for suitability[4] in assaying the antibacterial substance. This is done by adding from 10,000 to 1,000,000 bacteria to tubes of test media containing known concentrations of the antibacterial substance and 1.5 per cent agar and pouring into petri dishes. The number of colonies is counted after an incubation of 48 hours.

When *Staph. aureus* H. was found to be unsuitable for assay of streptothricin solutions, the reason was sought by the plating method outlined above. Two cells of every 10,000 were resistant to 20 times the inhibitory concentration of streptothricin and very many were resistant to twice the inhibitory concentration. The same culture contained in 40 million cells only 1 cell resistant to 2.5 times the inhibitory concentration of penicillin. In another test, there were only 20 cells per million resistant to twice the minimum inhibitory concentration of penicillin. This strain of *Staph. aureus* is known to be satisfactory for assay of penicillin. In any 1000 cells of a culture

[4] A satisfactory bacterial culture is one which produces a population most of whose members are inhibited by a concentration of an antibacterial substance less than twice as great as the concentration which causes an appreciable decrease in number. Whether or not a bacterial species is a satisfactory test organism depends upon the strain and the substance used.

of *Staph. aureus* H., the chance of finding one cell resistant to penicillin is very small and the chance of finding one resistant to streptothricin is large. Similar tests showed that *Staph. aureus* H. is unsatisfactory for assay of streptomycin.

Incubation Temperatures. All tests using *Photo. Fischeri* are incubated at 15° C. The tests with *B. mycoides* and *B. subtilis* are incubated at 30° C, though the sensitivity of *B. mycoides* and *B. subtilis* to the antibacterial substances is increased considerably by incubation at temperatures lower than 30°. The tests with all other bacteria are made at 36–37° C.

Glassware. The serial dilution tests are done in 12×75 mm. Kahn tubes. The pipettes are EXAX Blue Line 1 ml. serological pipettes graduated in units of 0.01 ml. All pipettes are cleaned with a nitro-sulfuric acid cleaning mixture, rinsed with water, and sterilized. The Kahn tubes are packed inverted in small baskets, sterilized, and stored in them. The tubes become scratched on the outside during the process of washing and have a limited life. While slightly scratched tubes can be used in the serial dilution tests, the arithmetic-series tests require unscratched tubes.

All glassware is sterilized by autoclaving at 120° C for 15 minutes.

Preparation of Sample. Since the growth of the bacteria may be inhibited by increased acidity of the nutrient solution and the activity of the antibacterial substance may be greatly influenced by the acidity of the medium, the pH of the samples should be adjusted to about pH 6 before assaying. The solutions to be assayed are diluted with B.E. broth or water until the activity is less than 32 dilution units. Of course, the amount of dilution will depend upon the sensitivity of the test organism to the substance being assayed and the concentration of this substance. Frequently it is necessary to assay solutions that contain large amounts of organic solvents. Saturated aqueous solutions of chloroform or ethyl acetate did not inhibit *Staph. aureus.* Concentrations of ethyl alcohol less than 5 per cent did not inhibit *Staph. aureus, B. mycoides, E. coli, Kleb. pneumoniae,* and *Photo. Fischeri.* However, there is always the possibility of synergism between the organic solvent and a subinhibitory concentration of the antibacterial substance. The solutions of the antibacterial substances must be prepared under aseptic conditions or sterilized before assaying. Acidic substances and neutral substances can be filtered through Seitz or sintered-glass filters without loss by adsorption. Thermostable basic substances, e.g. streptothricin and streptomycin, are sterilized by heating rapidly to boiling. This is the most convenient way to sterilize thermostable antibacterial substances. The "Swinny Filter"[5] is a very useful form of the Seitz filter because samples of between 1 and 5 ml. can be filtered with a loss of less than 0.5 ml.

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[5] Made by Becton, Dickinson and Co.

Principle of the Dilution-Tube Test. The principle of the test is very simple. Several dilutions of the antibacterial substance in tubes are inoculated with the test bacterium, incubated, and the lowest concentration of the substance which causes apparently complete inhibition of the growth of the bacteria is taken as the inhibitory concentration. From this concentration is calculated the activity of the active substance.

The tests are read usually after 16–18 hours, after 24 hours, and after 42 hours of incubation. The concentration of bacteria and the composition of the test-media are chosen so that good turbidity will develop in 16 hours except with *Myco. phlei* and *Myco. smegma*, which are incubated for 24 hours before the first reading is made. Whether or not a large change occurs on incubation beyond 16 hours depends upon the active substance and the strain of bacteria.

The time of incubation that gives reproducible assays depends upon both the antibacterial substance and the strain of bacteria and can be determined by repeated assay of the same solutions. Some substances may show at the end of 42 hours of incubation only one-eighth as great an activity as that at the end of 16 hours of incubation.

The tests to be described fall into one of two categories: in one, the concentrations of the active substance in the tubes form a geometric progression; in the other test, the concentrations form an arithmetic progression. The various modifications of the two types of tests will be discussed separately.

Geometric-Series Tests. *Single series—non-pathogenic bacteria.* Three modifications of the geometric-series tests are used. The test used with relatively non-pathogenic organisms, and therefore used for most of the assays, requires the minimum of manipulation. Racks that hold two or three rows of 10 or 12 tubes each are filled with sterile, unplugged 12×75 mm. Kahn tubes, and 0.5 ml. inoculated broth are added to each tube by a "Cornwall[5] Pipetting Outfit" or by a 5 ml. pipette. A 0.5 ml. volume of the sterile antibacterial substance is added to the first tube by means of a sterile 1 ml. serological pipette. The contents of the tube are mixed thoroughly and 0.5 ml. are transferred to the next tube. The contents of this tube are mixed, 0.5 ml. are transferred to the next tube, and so on to the end of the row. The solution of the antibacterial substance is diluted twice in each step as follows:

Tube No.	1	2	3	4	5	6	7	8	9	10
Dilution	2	4	8	16	32	64	128	256	512	1024

The racks of tests are put in the appropriate incubator and left for about 16 hours, after which they are removed, shaken to aerate the solutions and to suspend bacteria that may have settled to the bottom of the tube. The

number of clear tubes (tubes without the slightest trace of turbidity) is counted and the racks are returned to the incubator. The number of clear tubes is counted again after 24 hours and after 42 hours of incubation.

If, after incubation, the first five tubes are clear and the sixth turbid, the antibacterial solution is said to be active at a dilution of 32, or to have an activity of 32 dilution units per ml. when tested against the organism used in the assay. We have found that for most purposes, the activity of our solutions can be expressed in terms of the highest dilution that gives a clear tube. Tubes with incomplete inhibition are never used in computing activities unless the active substance is one which does not inhibit the growth of all of the bacteria. When it is available, a standard solution made from the pure substance is included in each set of assays, and the results are reported in terms of the standard.[6] Usually the pure substance is neither available nor necessary.

Single series—pathogenic and luminescent bacteria. Tests with pathogenic bacteria and *Photobacterium* are done in a 1 ml. geometric-series dilution test as follows: To the tubes are added 0.5 ml. of broth, the serial dilution is performed as above, and 0.5 ml. of inoculated broth are added to each tube. The racks of tubes are incubated, and the clear tubes are counted. The dilution for a tube is twice that given in the dilution scheme. Approximately the same number of bacteria are added to each tube in this procedure.

Since unplugged tubes are used, it might be thought that air-borne contamination of the tubes would be frequent. On the contrary, obvious contamination is rare even after 40 hours of incubation. The number certainly is no larger than the number contaminated when plugged tubes are used. The extra handling given the plugged tubes increases the chances of finger-borne contaminations. It is advisable to use plugged tubes if there is any danger of accidentally infecting laboratory personnel from spilled tubes.

Intermediate-Tube Series. A third modification of the geometric-series dilution test is the intermediate-tube test. For it, two or more 5-tube tests are performed, using two or more dilutions of the solution assayed. These dilutions are chosen so that they form an arithmetic progression, beginning with 1, in which the common difference is the reciprocal of the number of tests. The usual assay procedure is one in which two 5-tube tests are performed, using dilutions of N and 1.5 N, where N is a dilution of the solution to be assayed such that the endpoint (last clear tube) will fall within the range

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[6] The practice of reporting the activity of one substance in terms of an entirely different one as a standard is not to be recommended because the two substances do not necessarily behave in the same way. Values obtained on the same test solution at different times will not be the same, even though the activity of the test solution gives the same activity in terms of dilution, unless the activity of the standard is also the same at all times.

of a 5-tube test. With our antibacterial solutions, N has varied between 1 and 256 for *Staph. aureus* H. After incubation the two sets of tubes (one row in the rack) will present one of two pictures. Either the number of clear tubes will be the same in both tests or the second set (1.5 dilution) will have one clear tube less than the first set. How, then, is the test to be interpreted? Consider first a specific example in which 4 tubes are clear in the first set and 3 tubes are clear in the second set. The four tubes of the first set represent a dilution of 16, while the three tubes of the second set represent a dilution of 12. From the first set, it is learned that the solution is active at a dilution of more than 16 and less than 32, and from the second set, that is as active at a dilution of more than 12 and less than 24. For the sake of con-

TABLE 2. *Dilution Table for Intermediate Tube Test*

Tubes Clear, Dilution of 1

Tubes clear, dilution 1.5	0	1	2	3	4	5
0	2	2.5				
1		3.5	5			
2			7	10		
3				14	20	
4					28	40
5						56

venience, such a solution would be assigned a dilution of 20, a value midway between 16 and 24. Now consider a test in which 4 tubes are clear in both sets. The first set indicates a dilution of between 16 and 32, while the second set indicates a dilution of between 24 and 48 thus narrowing the dilution to some value between 24 and 32. For convenience, the average, 28, is taken. Table 2 gives the values of the dilutions for all possible 5-tube tests set up as above.

This test seems to be more involved than the geometric-series dilution test. What then is the advantage of it? The maximum systematic uncertainty of dilution in the geometric-series dilution test is 50 per cent, whereas in

this test it is 20 per cent. As we do it, very little more work is required for this method than for the geometric-series dilution test. The necessity for diluting the sample 1.5 is avoided by adding 1 ml. of broth to the first tube of the second set. When 0.5 ml. of sample are added, the required dilution of 3 in the first tube is obtained. Only one pipette is used for each row of 10 tubes.

This test is much faster and easier to do than the arithmetic-series test. Although the intermediate-tube test can be used to obtain assays, using bacteria which are not suitable for the arithmetic test, there is no substitute for a satisfactory test organism (see footnote 4).

Arithmetic-Series Tests. The arithmetic-series type of dilution test is a slight modification of the 2 ml. test described by McKee, Rake and Menzel (2, 12). In this test, volumes of a suitably diluted antibacterial solution ranging from 0.02 to 0.10 ml. in increments of 0.01, are added, by a Kahn, or, better, by a 0.2 ml. measuring pipette to empty 12×75 mm. Kahn tubes. Then 0.5 or 1 ml. of inoculated broth is added from a 5 ml. pipette or a "Cornwall Pipetting Outfit." After incubation, the highest dilution which gives a clear tube is taken as the activity of the solution. The dilution is obtained from the following scheme for the 1 ml. test:

Tube No.	1	2	3	4	5	6	7	8	9
ml. sample added	0.1	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.02
Dilution	11	12.1	13.5	15.3	17.7	21	26	34.3	51
Relative Conc.	.091	.082	.074	.065	.057	.048	.038	.029	.02

The systematic uncertainty varies from 10 per cent of the dilution for the first tube, to 19 per cent for tube 5 and to 39 per cent for the last tube. Hence the solutions are diluted before assay so that the first cloudy tube will fall between tubes 2 and 6. While neither the dilution increment nor the percentage of uncertainty is constant for this test, tests can be devised in which either is constant.

Since it is the concentration of the antibacterial substance in the solution that determines the inhibition of the test organism, a test designed so that the concentration difference between adjacent tubes is constant would be desirable. Such a constant difference in concentration test can be set up by keeping the total volume constant in the above arithmetic test. However, the volumes of broth must then be measured with a 1 ml. pipette graduated in units of 0.01 ml., a difficult and tedious procedure. The concentration difference in the 1 ml. or 0.5 ml. arithmetic-series test is sufficiently constant for most purposes and is much easier to do than the test in which the difference is exactly constant. Because of the errors possible in measuring the small volumes, the antibacterial solutions are diluted so that the end-point will occur between the first and sixth tube.

Results Obtained by the Three Test Procedures. Three types of assay procedures with three different degrees of uncertainty have been described. The arithmetic-series test presumably is the most accurate, and the values obtained by it will be considered to be correct. From the way in which the intermediate-tube test is designed, it must give inhibitory concentrations less than those given by the geometric-series dilution test.

What is the usefulness of the three tests? The geometric-series dilution test is used with solutions which may show activity between rather wide limits, and when a precision greater than 50 per cent is not needed. The results of the geometric-series dilution test are used to determine the dilution which will bring the activity within the range of the more precise intermediate-tube and the arithmetic-series test. The type of test should be chosen after considering the precision needed.

The results obtained by assaying the same solutions by the three test procedures are shown in table 3. All of the antibacterial compounds[7] had a

TABLE 3. *Minimum Inhibitory Concentrations in Micrograms per Milliliter Obtained by Three Dilution Methods.*

Substance	Bacterium	Type of test		
		Geometric	Intermediate	Arithmetic
Penicillin G	Staph. aureus H	0.031	0.018	0.021
Penicillin G	Esch. coli	28	16	16.9
Penicillin X	Esch. coli	14.6	8.4	12.5
Streptomycin[a]	Kleb. pneumoniae	0.125	0.071	0.069
Streptomycin CaCl₂[a]	Kleb. pneumoniae	0.125	0.071	0.069

[a] Concentrations computed in terms of free base.

purity of 90 per cent or better. The 0.5 ml. arithmetic test was used. Since population densities are not necessarily the same in 0.5 and 1.0 ml. of broth after an incubation period of 24 hours, comparison is made between 0.5 ml. arithmetic-series and 0.5 ml. geometric-series and intermediate-tube tests. This precaution is not necessary when an inhibitory concentration relative to a standard is being measured, since in that case the effect of volume of medium on the test will presumably be the same for unknown and standard.

The best agreement was not always between the arithmetic-series and intermediate-tube tests; it was not infrequently best, between the geometric-series dilution and the arithmetic-series tests. The reason for these dis-

crepancies is unknown but may be related to the details of the inhibitory action of the antibacterial substance upon the test bacteria.

ERRORS

Although millions of serial dilution tests on antibacterial substances have been performed, no discussion of the errors of mechanical and biological source has been published. It is easy to show that there are large pipetting errors in a geometric-series dilution test and that sometimes resistant organisms can cause significant errors. The latter errors will be discussed first.

In about one-half of the assays of streptothricin solutions with *Staph. aureus* H. by the geometric-series dilution method, one or more tubes showing a good growth of bacteria will be followed in the series by at least one tube showing no evident growth of the bacteria although it contained a lower concentration of streptothricin than the ones showing growth. Thus there are two endpoints in one test, one of which may be at a concentration of streptothricin from 4 to 16 times as great as the other. The bacteria in the out-of-place tubes will be found to be quite resistant to streptothricin. It is evident that the occurrence of out-of-line tubes at the high dilution end of the series of clear tubes could make the activity of the solution appear to be one-half or less of its real activity. The only satisfactory way to eliminate this error is to use a strain of bacteria which does not contain (or form) resistant forms.

Because it is not obvious, a serious error in the dilution tests is caused by errors in measuring the volumes of liquids. The errors, like the test, form a geometrical series, and a small error raised to the tenth power, as it is in a ten-tube geometric-series dilution test, becomes a large error. This can be demonstrated very simply by doing a ten-tube test with one pipette and repeating the test with ten pipettes. When this was done the end-point was the ninth tube in the first test and the eighth tube in the second test. Too frequently, if the dilution for the arithmetic-series test is computed from the activity obtained from a geometric-series dilution test in which the endpoint was the sixth tube or more, the range of the arithmetic-series test will be missed. Usually the dilution is too great. Both bits of evidence indicate that the geometric-series test shows the activity to be greater than it is. Where are the sources of error and how large are they?

Since antibacterial tests are no way to discover errors in diluting, another method was sought which would have high precision even after the test-solution had been diluted 1000 times. By doing a dilution test with 5 N HCl and titrating the acid in each tube with 0.1 N NaOH, the required precision was obtained. Water, 0.50 ml., was put in each tube with a measuring pipette and a geometric-series dilution test was performed, using a 1 ml. serological pipette. Racks of tubes and a tube of acid were also given

to two experienced technicians who used their own pipettes and their usual technique since they did not know that the test was not the usual antibacterial test. The results are given in table 4. By weighing the water re-

TABLE 4. *Actual Dilution in Geometric-Series Dilution Tests Obtained from Titration of Acid.*

Tube Number		1	2	3	4	5	6	7	8	9	10
Theoretical Dilution		2	4	8	16	32	64	128	256	512	1024
Actual Dilution	W.J.	1.98	3.62	7.15	13.5	25	54				
"	W.J.	1.92	3.74	6.95	13.2	25	45				
"	C.A.	1.90	3.7	6.7	13.5	24	24				
"	C.A.	1.91	3.7	7.0	13	26	42				
"	F.K.	1.93	3.52	6.9	12.5	21					
"	F.K.	2.14	4.02	7.53	14.1	25.7	49.2	95.7	184	337	660
Computed for pipette of	F.K.	1.94	3.76	7.30	14.2	27.5	53.4	103	200	387	750

moved, it was found that the dry EXAX Blue Line serological pipette used by F. K. removed 0.54 ml. when the liquid was drawn up to the 0.50 mark. The pipette delivered 0.53 ml. The pipette was found by the acid titration method to remove and deliver 0.53 ml. When 0.53 ml. is added to 0.50 ml., the dilution of the solution added is 1.94. Hence the dilution in each succeeding tube is 1.94 raised to a power equal to the number of the tube (see table 4).

The dilutions actually obtained in the first five tubes were nearly those computed for the pipette of F.K. and considerable deviation began at the sixth tube. The error in pipetting probably could be eliminated by removing 0.47 ml. instead of 0.50 ml. of sample each time. But there are two other errors that can not be so easily avoided. One is the error in measuring the volume of broth put into the tube by the automatic syringe. In the above samples, the water volumes were measured with an error of 0.01 ml. The automatic syringe used for measuring the volume of the broth can cause two errors in volume, one the systematic error resulting from incorrect setting of the length of its piston stroke, and the second, a random one, caused by leakage of the popett valves. In a series of ten successive measurements of a 0.5 ml. volume, the average deviation from the mean was 0.02 ml., the largest deviation was—0.06 ml. The negative errors were fewer and larger than the positive errors. The systematic error probably can be reduced to about 0.02 ml. by carefully weighing 2 ml. of solution delivered by the syringe. A systematic error of 0.03 ml. superimposed upon the random error causes an error of dilution equivalent to 1 tube in a 9- or 10-tube geometric-series dilution test. A 5 ml. measuring pipette was used to measure the 0.5 ml. quanti-

ties of broth with a mean volume delivered of 0.496 and an average deviation of 0.006 ml., an error in volume so small as to be insignificant.

Another error is a hidden one that makes the dilutions less than theoretical and is caused by adherence of high potency material to the wall of the pipette above the 0.50 ml. mark; this is finally washed down into a high dilution tube where it makes a large decrease in dilution. The small amount of liquid that adheres to the outside of the tip of the pipette also decreases the dilution. Thus the mechanical errors make substances assayed seem more active than they are by making the dilution in a tube considerably less than it really is. Positive error in measuring the volume of broth added by the automatic pipette increases the dilution and tends to offset the errors of pipetting. The reason dilutions computed from endpoints obtained in the range of tube 6 to tube 10 are in serious error is now obvious. Since we want to use the geometric-series dilution test because of its convenience, we either dilute the solutions assayed so that the endpoint falls within the first five or six tubes or we use the results only as a guide in diluting for the intermediate-tube test. A pipette calibrated to contain 0.50 ml. and 1.00 ml. would eliminate the pipette errors. Unfortunately such a pipette, calibrated from the tip, is not available.

For measurements of the volumes delivered by the Kahn, the 1 ml. pipette, and the 0.1 ml. pipette, the meniscus was set by mechanical means so that the error of setting was not greater than the width of a calibration line.

The volumes in the arithmetic test are measured with a 0.1 or 0.2 ml. measuring pipette graduated in units of 0.01 ml. or a Kahn pipette graduated in 0.001 ml. The volumes of a Kahn and a 0.1 ml. measuring pipette at steps of 0.05 ml. were measured by weighing the volumes of water delivered with the following results:

Range	0–0.05	0.05–0.10	0.10–0.15	0.15–0.20
volume, Kahn	0.0526 '	0.0486	0.052	0.0506
volume, measuring	0.051	0.050		

The accuracy of the Kahn pipette is not very great. If the 0.10–0.20 volume were used in the 1 ml. test, the dilution would be 10.7 as compared with the theoretical 11.0. If the 0.05 volume were measured into the sixth tube, the dilution would be 20.0 as compared with the theoretical 21.0. Neither volume is in error enough to make a one-tube difference. The error of 0.05 ml. in measuring the volume of the broth in the tube probably would not make a 1-tube difference in the test.

If the activity of the sample is reported in terms of a standard of nearly the same activity, the errors of dilution presumably will be the same for both the sample and the standard. Then, when the concentration of the anti-

bacterial substance in the sample is computed from the ratio of the activities of the sample and the standard and the concentration of the standard, the error resulting from mechanical imperfections will be relatively small.

SUMMARY

Several modifications are described of serial dilution methods suitable for quantitative measurement of concentrations of antibacterial substances. Selection of the bacteria and media for maintaining the stock cultures are described in detail. The activity of the antibacterial substance against three Gram-positive, four Gram-negative, and two acid-fast bacteria provides data useful in characterizing the antibacterial substance. The composition of the four test-media needed for the bacteria and the physical conditions for the tests are described. The manipulative details are given for an arithmetic-series dilution test and for two types of geometric-series tests. The value of each test is indicated. The errors in dilution tests resulting from errors in measurements of volumes are determined and ways to avoid them are suggested. More serious errors can be caused by bacteria—perhaps only a few cells—resistant to the antibacterial substance.

THE NEW YORK BOTANICAL GARDEN
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DIFFERENTIATION OF CARROT ROOT TISSUE
GROWN IN VITRO

MICHAEL LEVINE

The study of vertebrate tissues in culture media has given unquestionable evidence of the unlimited power of the cell to proliferate, to differentiate, and to assume physiological activity. While very young embryos of the chick, duck, and rabbit cultivated in vitro can grow, differentiate, and undergo definite tissue organizations, embryonic tissues in culture media have not been capable of reproducing the entire embryo. White (1939a,b), in his cultures in vitro of the tumor-like growths that occur on certain tobacco hybrids, reported first an unlimited proliferative power of this tissue, and later in the same year contended that the tissue cells differentiate into mature cell types, and furthermore form leaves not unlike those produced in situ on this type of overgrowth. White believes that these cultures offer proof of the concept that the plant cell is a totipotent elementary organism.

Carrot root tissue grown in vitro has been studied intensively by Gautheret and by Nobécourt. Cambial tissue of the carrot root when grown in vitro, Gautheret (1940a) holds, shows a definite proximal-distal polarity which disappears in repeated subcultures, and forms an undifferentiated tissue mass. Yeast extract, he finds, increases the rate of growth over the purely synthetic media. In another paper (1940b) on cambial tissue growth, he finds tissue differentiation so that the exterior-interior polarity of histogenesis is maintained. Gautheret (1942) contends that plant growth substances are not specific root forming substances, but at low concentration favor cell multiplication as well as cell growth. The rooting effect induced by these chemicals is a new physiological response to toxic levels of these agents. Nobécourt (1942) studied the factors necessary to the growth of carrot tissue callus and he believes that glucose and indole acetic acid are indispensable. Carrot roots form tubercles slowly, Nobécourt (1943) believes, when grown in nutrient media with thiamine, but grow rapidly and form a tissue mass in the presence of indole acetic acid. He concludes that thiamine is necessary to the growth of carrot roots while indole acetic acid is needed to form undifferentiated tissue masses.

In studying the effects of the carcinogenic hydrocarbons on plant tissue growth in vitro, two distinct lines of carrot root tissue clones produced not only roots, but fully differentiated stems and leaves. The history of these cultures is considered of interest, for it shows as far as can be learned

from the available literature that this is the first instance in which tissue cultures have produced complete morphological and physiological differentiation.

METHODS AND MATERIALS

The technique and material used in the preparation of the culture media followed that of White (1943). The modifications of the nutrients employed, suggested by Hildebrandt and his associates (1946) were also tried. The writer found that the initiation of growth of the fragments of the carrot roots and other plant tissues used was hastened by the use of a modification of Pfeffer's (P) nutrient solution to which the ingredients of Uspenski's (U) media were added together with thiamine, pyridoxine, and glycine in the same proportions used in White's (W) standard medium. The U medium alone, as given by White (1936), proved satisfactory. Growth of initial cultures was obtained quickly by the addition of 0.1 to 0.5 mg. per 100 ml. of indole acetic acid or a-naphthalene acetic acid to the nutrient media employed. All media used were made semi-solid by the addition of 0.5 to 0.6 g. per 100 ml. of thoroughly washed shredded agar agar in distilled water.

The tissue used was obtained from fresh or actively growing carrots by plunging a trocar into the root from a freshly exposed surface. The plugs of tissue so obtained were cut into pieces 2–3 mm. long and 1 mm. in diameter. The cultures were first started in 50-ml. Erlenmeyer flasks to which 10 ml. of media was added. From two to four fragments of the tissue were placed on the agar, and as they grew they were transferred to 125-ml. flasks with 25 ml. of media.

OBSERVATIONS

The two cultures of carrot root tissue designated $C7_1$ and $C8_1$ that showed differentiation of the tissue mass into roots, stems, and leaves were started on April 20 and June 10, 1946, respectively. The $C8_1$ culture started on the latter date was first to form stem and leaves on November 26, while the former culture $C7_1$ produced roots first and then formed a stem with two apical primary leaves about January 10 of the present year. This plantlet has now formed characteristic dentate, pinnatifid leaves and another one with ovate, primitive leaves has appeared. Another subculture of $C8_1$ series is at this writing forming stem and leaves.

The history of these cultures which follows fails to throw light on the specific factors which contributed to the differentiation. It is assumed that approximately perfect nutritional conditions have been obtained sufficient to permit the tissue mass to follow its normal development.

Parent culture 8 was grown from an inoculum removed from an actively growing carrot grown in the laboratory. After the plant was removed from

the soil, the leaves were removed and the carrot broken. The fresh exposed tissue yielded by trocar several small cylinders of tissue from the inner surface of the cortex. These were introduced into 50-ml. flasks with 10 ml. of Uspenski media to which 0.1 mg. per 100 ml. of α-naphthalene acetic acid was added. Of the four fragments of tissue planted, three became contaminated with bacteria. The fourth inoculum was transferred on 13 June 1946 to an Erlenmeyer flask of the same size and with media from the same batch first used. Growth was slow and uneventful and a tissue mass developed without the formation of any organs.

Cosmos, Bryophyllum, tomato, and marigold stems, when treated with α-naphthalene acetic acid, produce an abundance of roots (Levine 1936). Here, this substance elicited no macroscopic response from the culture. On 26 July 1946 the culture was photographed through the flask and showed about 12 fused, nodular bodies which comprised the tissue mass. The color of the tissue was slightly tinged with green. On September 8 the tissue mass was divided into five parts. Culture 8_1 was planted on W; $C8_2$ was transferred to U medium to which 0.5 mg. per 100 ml. indole acetic acid was added; and $C8_3$–$C8_4$ and $C8_5$ were planted on the W media with certain concentrations of methylcholanthrene, benzpyrene, and dibenzanthracene.

The purpose of this experiment which forms a part of the whole investigation, was to study the influence of the carcinogenic agents on plant tissue supplied with adequate nutrient materials. The growth-stimulating substance was added so as to induce cellular changes which might make the cells more susceptible to the influence of the carcinogens.

Culture 8_1 grew rapidly and on 12 October 1946 two roots made their appearance from the upper surface of the tissue mass. Shortly thereafter and unexpectedly, a thin narrow ribbon-like stem and leaf-like organs appeared. The edges of these organs were magenta in color, while the rest of the leaves were colorless.

On November 26, the first stem-like structure assumed an erect position. It failed to produce leaves, but its apical portion became blade-like. The other leaf-like structures (fig. 1) seemed to arise from a short stem which developed from the lateral surface of the tissue mass. The roots were well formed and apparently normal. Subsequent development showed evidence of fasciation and can be seen in a photograph (fig. 2) made of the growth while in its glass container on 6 December 1946. This plant is aberrant. It never developed sufficient chlorophyl to function, but there is no question of complete morphological differentiation of this tissue into root, stem, and leaves. The fusion of parts of the plantlet failed to set off the stem. A leaf and a root were fixed for microscopical examination.

Culture 8_1 was divided on 12 December 1946 and $C8_{1\ 5}$ was started on W media with a fragment of the tissue mass. The explant grew slowly and on
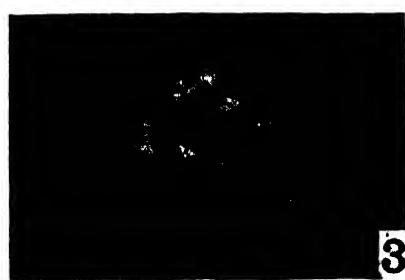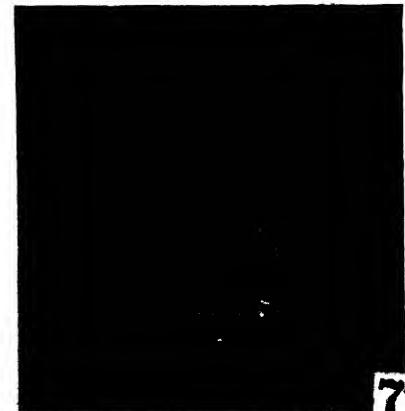
30 January 1947 showed the early development of several plantlets. There are only a few roots formed not shown in the photograph (fig. 8). There is no evidence of fasciation; however the culture resembles the early development of the plantlets of $C7_1$ described below.

The contention that fasciation is of bacterial origin is not supported by these bacteria-free tissue cultures. The causes of fasciation may be sought in the initial tissue used and may possibly be associated with injury. In this case the injury may have been induced at the time the tissue mass was divided.

Carrot root culture 7_1, which gave rise to two fully differentiated plants and several that are in the process of development was started on 20 April 1946 on U medium. Two inocula used in the parent culture were obtained from the root meristem of a small spring carrot. The inocula measured 2 mm. × 1 mm. and both were immersed for a few seconds in a sterile solution of 0.1 mg. per 100 ml. of α-naphthalene acetic acid and then transferred to a 125-ml. flask with 25 ml. U medium. On 1 May 1946 both inocula showed evidence of growth, and before these cultures were a month old (May 13) they had more than doubled their size. On June 7 the two inocula were separated and each was placed in a medium consisting of P nutrient solution together with 4 mg. of glycine, 1 mg. of thiamine, 1 drop of Beminal, and 6 g. of well washed shredded agar, made with 1 l. of distilled water. It appeared from other cultures in progress that the beneficial effects of the Beminal were due to pyridoxine. On June 24, $C7_1$ and $C7_2$ were transferred to P U medium to which was added 5 mg. per 100 ml. of vitamine B_6.

On October 22 $C7_2$ developed into an abundant tissue mass with roots as shown in figure 3. $C7_2$ was divided into 10 subcultures on October 23. $C7_2$–8 grown on W to which was added 0.1 mg. per 100 ml. α-naphthalene acetic acid, is shown in figure 4, photographed December 14. No stem nor leaves were produced. The other subcultures were treated with carcinogenic hydrocarbons.

Explanation of figures 1–8

FIGS. 1–8. From cultures in vitro of the meristem of carrot roots. FIG. 1. Culture 8_1 started 6.10.46, photographed 11.26.46. See text for complete record of growth and transfer. FIG. 2. Same culture on 12.6.46, note fasciated stem and leaves. FIG. 3. Culture of carrot 7_2 started from a fragment of carrot root meristem 4.20.46, photographed 10.22.46, showing only roots arising from upper surface of tissue mass. FIG. 4. Subculture 8 of 7_2 photographed 12.14.46. Note the increased number of roots, no leaves. FIG. 5. Culture 7_1 started with 7_2, photographed 1.23.47, with complete differentiation of tissue mass into roots, stems, and leaves. Note small plant just above water condensed on flask. FIG. 6. The same culture showing several plantlets with well differentiated roots, stems, and leaves, photo 1.28.47). FIG. 7. The same culture on 1.30.47. Note development of two young plants from the tissue mass. FIG. 8. Clone of C8, divided 12.12.46. Shows early stages in differentiation of tissue mass. The structure first appeared 1.20.47, photographed 1.30.47.

On 6 January 1947 the tissue mass of $C7_2$–8 was divided into 9 fragments. $C7_2$–8_5, grown on W nutrient medium to which 1,2,5,6-dibenzanthracene had been added in proportion of 0.1 mg. per 100 ml. of media, grew slowly. On January 23 evidence of differentiation of roots, stem, and leaves appeared. The roots were numerous and apparently normal, while the three stems formed were small. Their first leaves were minute and difficult to separate. The color of these aërial organs is a pale greenish-yellow. The tissue mass from which these plantlets developed is not abundant. The entire culture growth, by comparison, seems to be inhibited. A sister culture 7_2–8_6 was transplanted to W to which 0.2 mg. per 100 ml. of 3,4-benzpyrene had been added. The growth resulted in a fairly active tissue mass with roots and several stem-like structures. The latter may be aërial roots, for no leafy elements are distinguishable. Usually by the time the stem becomes erect, the apical region bears the characteristic leaflets.

Culture 7_1 after the transfer of June 24 mentioned above, grew but slightly and on July 26 was transferred to P U with 0.1 mg. per 100 ml. of a-naphthalene acetic acid, and again on July 30. Growth of the tissue became active again and on September 16 the tissue mass was divided into 18 parts. One part of the original $C7_1$ tissue mass remained in the flask and it was noted that it had grown luxuriantly, and on December 13 it was transferred to W. After transfer of the tissue mass, growth was slow, yet several roots began to appear. About the first week in January, 1947, a single pale yellowish-green-colored stalk appeared at the base of the tissue mass near the surface of the agar. The tip of the stem was bent toward the agar, but in several days it became erect and terminated in two oval, primitive leaves. Figure 5 was photographed on January 23. The stem and leaves became green, and minute whitish bud-like structures, which formed on the stem below the terminal leaves, developed into a typical pinnate leaf characteristic of *Daucus carota*. Another plantlet that arose from the same area of the tissue mass is shown in figure 6 (photographed 28 January 1947), but it retains a pale greenish color with specks of red. Figure 7 shows the development of the plantlets in $C7_1$ on January 30. The tissue mass was not observed to increase in size. Here is evidence of the transformation of a fragment of carrot root tissue grown in vitro into a tissue mass from which a morphological differentiation of functioning root, stem, and leaves occurs. The cellular structure of the tissue mass is under investigation.

<center>DISCUSSION</center>

The cell masses used in these experiments were derived from minute plugs of tissue removed from the meristem of the root of a carrot. This tissue does not express its inherent capacity for differentiation into the principal organs of the plant while in situ on the plant. These experiments fail to

reveal any conditions necessary to the differentiation of this tissue, except an adequate nutritional basic medium to which a growth stimulant, α-naphthalene acetic acid, was added. The presence of this stimulant is apparently needed to initiate rapid growth.

Cultures to which the carcinogenic hydrocarbons had been added indicate clearly that, given a suitable nutrient medium, the addition of the carcinogen does not deter cell mass differentiation. This suggests that the growth substance does not induce differentiation. The nature of the tissue determines its capacity to differentiate. The plant growth substance facilitates the development and differentiation follows more quickly and more completely. The differentiation of the tissue mass does not occur in the initial culture, but only after few passages made over a relatively brief period.

White has shown (1939a, b) that a tissue culture of the callus overgrowths of the tobacco hybrid are not only unlimited, but he contends that under conditions of reduced oxygen tension and after many passages over a period of a year and a half leaves will be produced. No stems nor roots were observed in his cultures. The calloid tumors of the tobacco hybrid grown in the garden have been shown (Levine 1937) to consist of nodular masses covered with leafy structures. There is no tendency of this overgrowth in situ on the hybrid to form roots. It appears that the callus tissue has a tendency to produce leafy structures and that submerging the cell mass and so reducing the oxygen tension enables the potentialities of the tissue to emerge. This would suggest the presence of a predetermining factor to form leaves, inherent in this tissue. The meristem tissue of the carrot root differs from the callus tissue of the tobacco hybrid in that there is no evidence of a determining factor which will cause the meristem to produce a plant organ, but its totipotency enables it under an adequate environment to differentiate into the three fundamental organs of the plant.

SUMMARY

Cultures in vitro of carrot meristem, grown on various media to which the plant growth substance indole acetic acid or α-naphthalene acetic acid is added, produce a tissue mass.

In several instances tissue masses grown in a suitable medium to which α-naphthalene acetic acid was added produced complete morphological and physiological differentiation of the tissue into root, stem, and leaf.

Clones of these tissue masses grew and formed plantlets when transferred to W medium to which one of the carcinogens used here was added. A report on this phase of the work is in progress.

Atypical, fasciated plantlets have been observed in these in vitro cultures.

All cultures were grown on semi-solid media and no tissue mass was completely submerged in it.

I am indebted to Dr. Emil J. Baumann for his kindness in the preparation of the media used in these studies.   Thanks are due Mr. G. Keller for help given in connection with the photographs shown here.

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A NEW ERIOGONUM FROM THE SOUTHEAST

GEORGE J. GOODMAN

Eriogonum Harperi Goodman, n. sp. Planta perennis, erecta, 1 m. vel plus alta; caulibus plerumque ad inflorescentiam non ramosis, molliter griseo-tomentosis; foliis basalibus longe ellipticis, lamina ca. 15 cm. longa, infra dense tomentosa, supra glabra vel parce pilosa; foliis caulinis usque ad 1 dm. longis, 2 cm. latis; involucris 3–3.5 mm. longis; floribus 4–5 mm. longis, pilis ascendentibus vestitis; ovario pubescente, achaenio maturo exserto, ad apicem 3-alato.

Perennial taprooted plants, erect, 1 m. or more tall; stems mostly unbranched to the inflorescence, softly gray-tomentose; basal leaves long-elliptic, blades about 15 cm. long, 2.5 cm. wide, densely tomentose below, glabrous to scantily pilose above, narrowed to a petiole; stem leaves alternate, up to 1 dm. long and 2 cm. wide, gradually diminishing in size upwardly, elliptic, acute, irregularly revolute, at least in drying, giving a repand appearance, tomentose below, scantily short-pilose above; axillary buds frequently developing into leaf clusters; inflorescence paniculate, the frequently pedunculate involucres secund, bracts ternate, involucres 3–3½ mm. long, the teeth erect, triangular, flowers many per involucre, 4–5 mm. long, covered with ascending hairs, segments oblong-elliptic; stamens 9, filaments glabrous, anthers oblong, 1–1.2 mm. long, ovary pubescent, mature achene exserted, 3-winged toward apex.

Type: Marly glade in saddle on Little Mountain, about 3 miles south of Tuscumbia, Colbert Co., Alabama, August 12, 1943, *Roland M. Harper 3944.* (Type in Bebb Herbarium, University of Oklahoma.)

This species differs from *Eriogonum longifolium* in its broader stem leaves which are more abundant up to the inflorescence, the glabrous or nearly glabrous upper surface of the leaves, the smaller involucres and flowers, the shorter and less abundant hair on the calices, and the achenes, which are 3-winged instead of 3-angled. It is also out of the known range, by 300 miles, of *E. longifolium* and is 500 miles from its other relative, *E. floridanum*. *E. floridanum* has even larger involucres and flowers than *E. longifolium*.

Ample material of this species, consisting of specimens of basal leaves, the flowering plant, photographs, and field notes, was generously sent me by the discoverer, Professor Roland M. Harper, University of Alabama. It is a pleasure to dedicate the species to him. Some of his notes tell more of the plant's range and habit. The species was first observed by Prof. Harper as a single specimen in a calcareous prairie area about one mile east of Littleville, Colbert County, on July 25, 1942. On October 7 of that year on Little Mountain, in the same county, four over-ripe specimens were collected. Photo-

Fig. 2.

Fig. 3.

graphs of the fruiting calyces of these were obtained. On August 12, 1943, several specimens—the type collection—were collected in bloom at this same location. In March of 1944 the species was found in a small cedar glade near Russellville, a few miles from the other stations in adjacent Franklin County. Although Professor Harper is not yet certain, he believes that the plant blooms but once.

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Explanation of figures 1–3

FIG. 1. Flowering branch of type (× 1). The downcurved tips of some of the branches are the result of wilting. FIG. 2. Habit of type collection. FIG. 3. Harper 3902, collected Oct. 7, 1942, showing general aspect, taproot, and basal leaves.

EUPHORBIA MACULATA AGAIN

F. R. FOSBERG

It seems necessary, even at the risk of unduly prolonging this discussion, to point out the curious slip in Croizat's reasoning (Bull. Torrey Club **74**: 153–155. 1947) in his persistent attempt to apply the name *Euphorbia maculata* L. to the prostrate plant now known as *Euphorbia supina* Raf.

In taking me to task for upholding Wheeler's interpretation of this matter. (Contr. Gray Herb. **127**: 76. 1939; Rhodora **48**: 197–200. 1946) Croizat uses an argument which might, in another case, be convincing enough. He shows that Boissier (DC. Prodr. **15²**: 46. 1862) selected one of two elements in the original Linnaean concept of this species, and that the element selected was what I referred to *E. supina*, on the basis of one of the two specimens in the Linnaean Herbarium. He regards this as "an iron-clad 'typification' of the most modern pattern" and considers that this settles the case. On p. 154 he attempts to strengthen his case by referring to the one Linnaean specimen, agreeing with "Fosberg's *E. supina* Raf." as "inscribed '*21. maculata*' in an handwriting which is to all appearances Linnaeus' own," and to the other ("my" *E. maculata* L.) as "inscribed in different handwritings both '*maculata*' and '*hypericifolia*,'" and leaving the reader to the inference that neither of these handwritings are that of Linnaeus. It is not necessary to dispose of this matter.

The point that Croizat missed is that Linnaeus, in the *Mantissa* (**2**: 392. 1771), by obviously referring to the *hypericifolia*-like element in the concept, had already typified his species in as "iron-clad" a fashion as could be desired. Even Boissier tacitly recognized this when he felt it necessary to exclude the concept adopted in the *Mantissa*. Though the rules are silent on the question of whether the earliest typification must stand, sound nomenclatural philosophy necessitates it unless very conclusive reasons exist for retypification.

That no such reasons exist in this case may be seen by examination of the treatment of the species in the *Species Plantarum* (ed. 1. 455. 1753) where are presented a reference to a previously published plate and a description of the plant. The plate I have shown (Rhodora, l.c.) to portray the erect plant rather than the prostrate *E. supina*. The description, while less conclusive, more easily fits the erect plant than the prostrate one because of the mention of trinerved, serrate leaves, two characters that are much more

prominent in the erect than the prostrate plant, and the lack of reference to a prostrate habit. This description, by the way, shows that, in the very case he is discussing, Croizat is in error in referring to the *Species Plantarum* as "an outright compilation," though it certainly is in most parts.

Thus, it appears that Croizat's very principle of prior typification has, in this case, defeated his argument and that the use of the binomial *Euphorbia maculata* L. "in the Wheelerian manner," for the upright plant, is the correct use.

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NEW SPECIES OF CHYTRIOMYCES

JOHN S. KARLING[1]

The genus *Chytriomyces* was created to include two saprophytic eucarpic, monocentric, operculate chytrids which were found by the author (1945) on bits of insect skeleton in fresh water in equatorial Brazil. Subsequently, these species were found to occur abundantly in muck soil and fresh water in Virginia, New Jersey, New York, Connecticut, and Massachusetts. A third species, *C. nodulatus*, was found by Haskins (1946) in England, and recently Miss Fay (1947) described another species, *C. spinosus*, from New Jersey. Species of this genus, therefore, appear to be widely distributed in nature and occur chiefly on substrata which contain chitin. Accordingly, these chytrids were first described as being chitinophilic, but it has been shown subsequently by Miss Fay, Haskins, and the author (1947) that they will grow moderately well also on substrata which contain cellulose and keratin.

The present contribution concerns three additional species of *Chytriomyces* which have been found in Brazil and the eastern part of the United States. One of these species parasitizes *Aphanomyces laevis*, causing local swelling on the mycelium and excessive branching. It is, accordingly, named *Chytriomyces parasiticus*. The other two are saprophytic on chitinous substrata in muck soil and freshwater. One of these species is very large and distinguishable by appendiculate sporangia, while the other one is characterized primarily by stellate resting spores. They are, therefore, named *C. appendiculatus* and *C. stellatus*, respectively. The type and general method of development of these species are fundamentally similar to those described previously for members of *Chytriomyces*, and it is not necessary to discuss these processes again. The present paper will be limited, accordingly, to the outstanding structural characteristics.

Chytriomyces parasiticus Karling, sp. nov. Fungus parasiticus. Sporangiis laevibus, hyalinis, sphaericis, 8–30 μ diam., aut ovalibus; operculo apicali aut subapicali, 4–14 μ diam. Zoosporis ovalibus, 2.5–3 μ diam., globulo refractivo hyalino, 0.4–.6 μ diam.; flagello 14–18 μ longo. Apophysis globulis, 3–6 μ diam., aut angulata, intra- vel extramatricalis. Sporae perdurantes ignotae.

Sporangia smooth, hyaline, spherical, 8–30 μ, or slightly oval; operculum apical or subapical, 4–14 μ diam. Zoospores oval, 2.5–3 μ, with a minute, 0.4–6 μ diam., hyaline refractive globule; flagellum 14–18 μ long. Apophysis

intra- or extramatrical, globular, 3–6 µ diam., or angular; rhizoids relatively short and finely branched. Resting spores unknown.

Parasitic on *Aphanomyces laevis,* New York City, causing local swelling and excessive branching of the mycelium.

Chytriomyces appendiculatus Karling, sp. nov.   Fungus saprophyticus. Sporangiis appendiculatis, laevibus, subfuscis, rare sphaericis, 10–80 µ diam., aut ovalibus, 10–50 × 30–90 µ diam., aut oblongatis, 10–20 × 30–50 µ diam., aut pyriformibus, 20–180 × 35–250 µ diam., aut reniformibus, irregularibus, aut rare lobatis; parietibus crassiusculis, 1–3 µ; operculo apicali aut subapicali, 6–14 µ diam., hypocrateriformi demum evanescenti. Zoosporis ovalibus, 4–5 × 6–6.5 µ diam., globulo refractivo hyalino, 1.8–2.8 µ diam.; flagello 28–32 µ longo. Hyphis rhizomorphis ramosis, crassiusculis, 3–18 µ diam., aut tenuissimis. Sporis perdurantibus plerumque appendiculatis, laevibus, sphaericis, 10–25 µ diam., aut ovalibus, 10–15 × 18–24 µ diam., aut irregularibus; parietibus crassiusculis, 2.5–5 µ, fuscis; germinantibus zoosporangia tenui membranata superficialia genertibus.

Sporangia appendiculate, smooth, hyaline when young, but usually becoming brown with age, highly variable in size and shape, rarely spherical, 10–80 µ, flattened or oval, 10–50 > 30–90 µ, oblong, 10–20 × 30–50 µ, irregularly pyriform, 20-180 × 35-250 µ, slightly bean-shaped, tilted, irregular and lobed with a 1–3 µ thick wall; operculum non-persistent, shallow saucer-shaped, 6–14 µ diam. Zoospores oval 4–5 × 6–6.5 µ, with a conspicuous, 1.8–2.8 µ diam., hyaline refractive globule; flagellum 28–32 µ long. Rhizoids coarse, main axes up to 18 µ in diam. in large thalli, branched, usually becoming thick-walled with age. Resting spores smooth and usually appendiculate, spherical, 10–25 µ, oval, 10–15 × 18–24 µ, predominantly irregular with a 2.5–5 µ thick, brown wall; content coarsely but evenly granular with a central vacuole, emerging through a pore in the wall during germination and forming a superficial zoosporangium.

Saprophytic on chitinous substrata in freshwater and muck soil, Virginia, New Jersey, New York, and Connecticut.

Chytriomyces stellatus Karling, sp. nov.   Fungus saprophyticus. Sporangiis hyalinis laevibus, 1–6 brevibus triangularibus clavis spinisve instructis, sphaericis, 9–50 µ diam., aut ovalibus, 8–35 × 10–45 µ diam., aut pyriformibus, 6–20 × 14–38 µ diam., aut reniformibus, aut rare angularibus, 1–3 papillatis aut tubulo, 3–7 × 5–50 µ diam., exeuntibus. Zoosporis ovalibus, 3.5–4 × 4.5–5 µ diam., globulo refractivo hyalino, 1.5–2 µ diam.; flagello 25–30 µ longo. Hyphis rhizomorphis e apophysis basi vel lateribus ortis, ramulosis. Sporis perdurantibus hyalinis, apophysatis, sphaericis, 9–26 µ diam., aut ovalibus, 10–14 × 15–22 µ diam., aut angularibus, paucis vel multis, obtusis vel elongatis, acutis vel rare bifurcatis chavis spinisve instructis, rare brevibus; globulo usque ad 10 µ diam., vel nonnullis minoribus globulis refractivis; germinantibus zoosporangium e parietis poro ortum tenui membranatum genertibus.

Sporangia hyaline, smooth or with 1–6 short, solid, triangular pegs or spines, spherical, 9–50 µ, oval, broadly pyriform, 8–35 × 10–45 µ, narrowly pyriform, 6–20 × 14–38 µ, bean-shaped, anatropous or slightly angular with 1–3 exit papillae or long, 3–7 × 10–50 µ, necks; operculum saucer-shaped

or slightly apiculate, 4–7 μ diam. Zoospores oval, 3.5–4 × 4.5–5 μ, with a small, 1.5–20 μ diam. hyaline refractive globule; flagellum 25–30 μ long. Apophysis conspicuous, spherical, 4–18 μ, oval, 4–8 × 6–14 μ, broadly fusiform or slightly angular. Rhizoids arising from the base or sides of apophysis, finely branched. Resting spores hyaline, apophysate, spherical, 9–26 μ, oval, 10–14 × 15–22 μ, or slightly angular with few to numerous solid, short blunt or elongate, pointed, and rarely bifurcate pegs or spines, rarely smooth; content hyaline with one large, up to 10 μ in diam., or several smaller, refractive globules, emerging through a pore in the wall and forming a superficial zoosporangium during germination.

Saprophytic on chitinous substrata in freshwater and muck soil, São Carlos, Matto Grosso, Brazil; New York and Connecticut.

CHYTRIOMYCES PARASITICUS. This species occurred on the mycelium and filamentous, undifferentiated zoosporangia of *Aphanomyces laevis* which had been collected on bits of purified shrimp chitin in water from Van Cortlandt Park, New York City. After being brought into the laboratory, the host became so heavily parasitized, that most of its mycelium was killed in the course of two weeks, and by the end of a month the epidemic had run its course and no trace of the parasite could be found.

As shown in figure 1, this parasite causes marked local hypertrophy of the host mycelium and excessive branching in the region of infection. In a few cases, as many as 30–80 short branches have been observed at the site of infection. Quite often the sporangium of the parasite may be partly enveloped and obscured by the branches, as shown in figures 1A, 1B, and 6. However, branching does not always follow infection, and the reaction of the host may be limited to the development of broadly fusiform and globular swellings (figs. 1C, 1D, 1E). The effect of the parasite on the host protoplasm is quite marked and easily recognizable. After the zoospore has come to rest on the mycelium and developed an infection tube, the host protoplasm begins to accumulate in the region of infection (figs. 1F, 1G, 1H, 4). It soon becomes more heterogeneous optically and coarsely granular. As the absorbing system or rhizoids develop from the germ tube, the protoplasm becomes more densely aggregated around the rhizoids, and by the time the parasite is mature a large part of it has been absorbed from the swelling.

The thallus of *C. parasiticus* may be apophysate (figs. 1C, 1E) or non-apophysate (figs. 7, 9) like *C. aureus* and *C. hyalinus*. When present, the apophysis is usually intramatrical, but occasionally it may be extramatrical or partially so (fig. 8). In shape it usually appears angular or digitate because of the rhizoids which arise from several points on its surface (figs. 1D, 1E, 10). Its exact size and shape are difficult to determine because of the surrounding host protoplasm. The rhizoids are comparatively short but finely branched, and, like the apophysis, they are usually obscured by the host protoplasm.
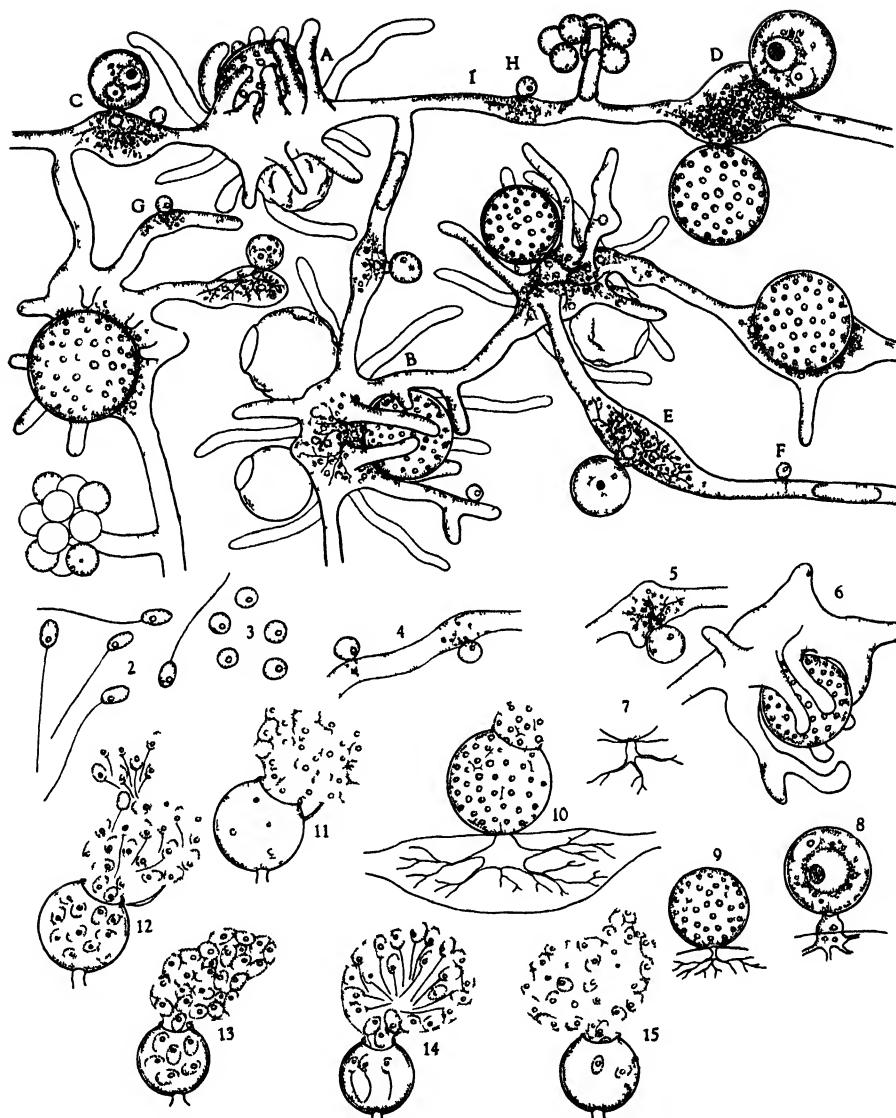
FIGS 1–15 *Chytriomyces parasiticus* FIG 1 Mycelium of *Aphanomyces laevis* parasitized by 20 thalli of *C parasiticus* in various stages of development, showing local hypertrophy and excessive branching in the regions of infection FIGS 2, 3 Motile and quiescent zoospores of parasite FIG 4 Initial stages of infection and reaction of host FIGS 5, 6 Later, successive stages of development of the parasite shown at the right in figure 6 and reaction of the host FIG 7 Rhizoids of non apophysate thallus FIG. 8 Apophysate sporangium with large primary nucleus and oval vacuole FIG 9 Late developmental stage of non apophysate thallus FIG 10 Emergence of zoospores FIGS 11, 12 Zoospores swarming in external vesicle and escaping after rupture of vesicle FIGS 13–15 Successive stages of swarming and quiescence of zoospores in external vesicle

Like all other species of *Chytriomyces* studied by the author, *C. parasiticus* possesses a large primary nucleus which can be readily seen in living sporangia (figs. 1C, 1D, 1E, 8). It appears as a relatively clear sphere with a large nucleole and is usually surrounded by a dense layer of cytoplasm in which numerous granules and globules are suspended. The nucleus keeps pace with the growth of the sporangium and apparently does not begin to divide until the latter has attained its definitive size. Quite often a large conspicuous vacuole is present in the cytoplasm (figs. 1C, 1D, 8) and contains a minute body which undergoes rapid Brownian movement.

The zoospores of *C. parasiticus* are minute and oval in shape and contain a small refractive globule (fig. 2). During the swimming period, they dart and hop about like those of *Rozella* species. They dart about for a distance of from 60 to 150 μ, then come to an abrupt and complete stop, and then dart off again. The intermittent pauses are so brief, however, that it is almost impossible to determine the shape and structure of the zoospores in the active stage. At the time of dehiscence they emerge slowly from the sporangium (fig. 10) and form a globular mass at the exit orifice. After 30–80 seconds after emerging they begin to move about slowly at first but in a short while they swarm rapidly within a hyaline vesicle which is continuous with the sporangium (fig. 11). This swarming period lasts from 1 to 3 minutes after which the vesicle ruptures and the zoospores escape (fig. 12). This normal behavior of the zoospores is identical to that described by the author for *C. aureus* and *C. hyalinus*.

Variations of behavior, however, have been observed in a few cases. After swarming rapidly in the vesicle for 2 minutes (fig. 13), the zoospores came to rest for a period of 20–35 seconds. In this resting stage they were generally disposed at the periphery of the vesicle with their flagella extending towards the center, as shown in figure 14. Then followed another swarming period which lasted from 45 to 70 seconds (fig. 15), after which the zoospores came to rest in much the same manner and position shown in figure 14. This was followed by a third swarming which ended with the rupture of the vesicle. Thusly, the zoospores of *C. parasiticus* may undergo several intermittent periods of swarming if the vesicle fails to rupture at first, and these swarmings are comparable to the diplanetism exhibited by the zoospores of the Oomycetes, with the exception that the flagella are not retracted and no cystospores are formed.

So far, no resting spores have been found in this species, so that its life cycle is only partially known. However, the character of the operculate, extramatrical sporangia and the behavior of the zoospores warrant the classification of this fungus as a species of *Chytriomyces*.

CHYTRIOMYCES APPENDICULATUS. This species was isolated first in 1944 from swamp soil collected at Mt. Prospect, Va., and later found in New

Jersey, New York, and Connecticut. It grows readily on insect skeletons
and purified chitin, and on the latter medium the sporangia may become
so numerous that they impart a dull brown color to the substratum. From
strips of chitin it is readily transferable to chitin agar, on which it may be
grown in pure culture. The sporangia usually become brown in color when
grown on chitin in spring or brook water, but on chitin-agar they remain
hyaline. Inasmuch as the former environment is the more natural and the
one under which the sporangia are brown in color, they are, accordingly,
described as non-hyaline in the diagnosis above.

Chytriomyces appendiculatus is primarily distinguishable by appendic-
ulate sporangia which are highly variable in size and shape. Some of its
larger sporangia may be 250 μ in diameter, and in this respect it is the
largest known species of *Chytriomyces*. The appendage to the sporangium
begins usually as a thickening of one side of the wall of the germinated
zoospore (fig. 20) As a result, expansion and growth appear to be retarded
on that side, and as the incipient sporangium increases in size, it expands
at the opposite side and becomes asymmetrical, as shown in figures 21 and
22. With further growth of the sporangium and development of the thallus,
the thickened portion of the wall may become further modified and thick-
ened into variously shaped appendages (figs. 22, 25, 28, 30, 31, 32) which
usually turn brown with age In other cases, the wall of the germinated
spore or incipient sporangium thickens more or less equally (fig. 23) with
the result that sporangia with uniformly thick walls are formed (figs. 24,
25, 26) Figure 26 shows an immature, slowly growing or dormant spo-
rangium filled with granular and refractive protoplasm, which is not un-
common in this species. The wall is fairly uniform in thickness and dark
brown. When such sporangia resume growth and form zoospores, they
usually burst out of the thick wall and form a zoosporangium of the type
shown in figure 27. As a rule, small sporangia which develop rapidly and
form zoospores within a short period of time are uniformly thin-walled and
hyaline.

As in the previous species, the primary nucleus of the sporangium is
usually visible in living material as a large, clear, globular body in which
lies a large nucleole (figs. 22, 25). However, it is not clearly visible in large
sporangia which are vacuolate (fig. 29) or filled with granular and refrac-
tive material (fig. 28). Therefore, it has not been possible to determine in
living material whether or not it divides before the sporangium reaches its
mature size.

One of the striking characteristics of most mature sporangia of *C. ap-
pendiculatus* is the large amount of homogeneous hyaline slime or matrix
beneath the operculum. It is often greater in amount and more conspicuous
than that present in some sporangia of *C. hyalinus* (fig. 38) and *C. spinosus*
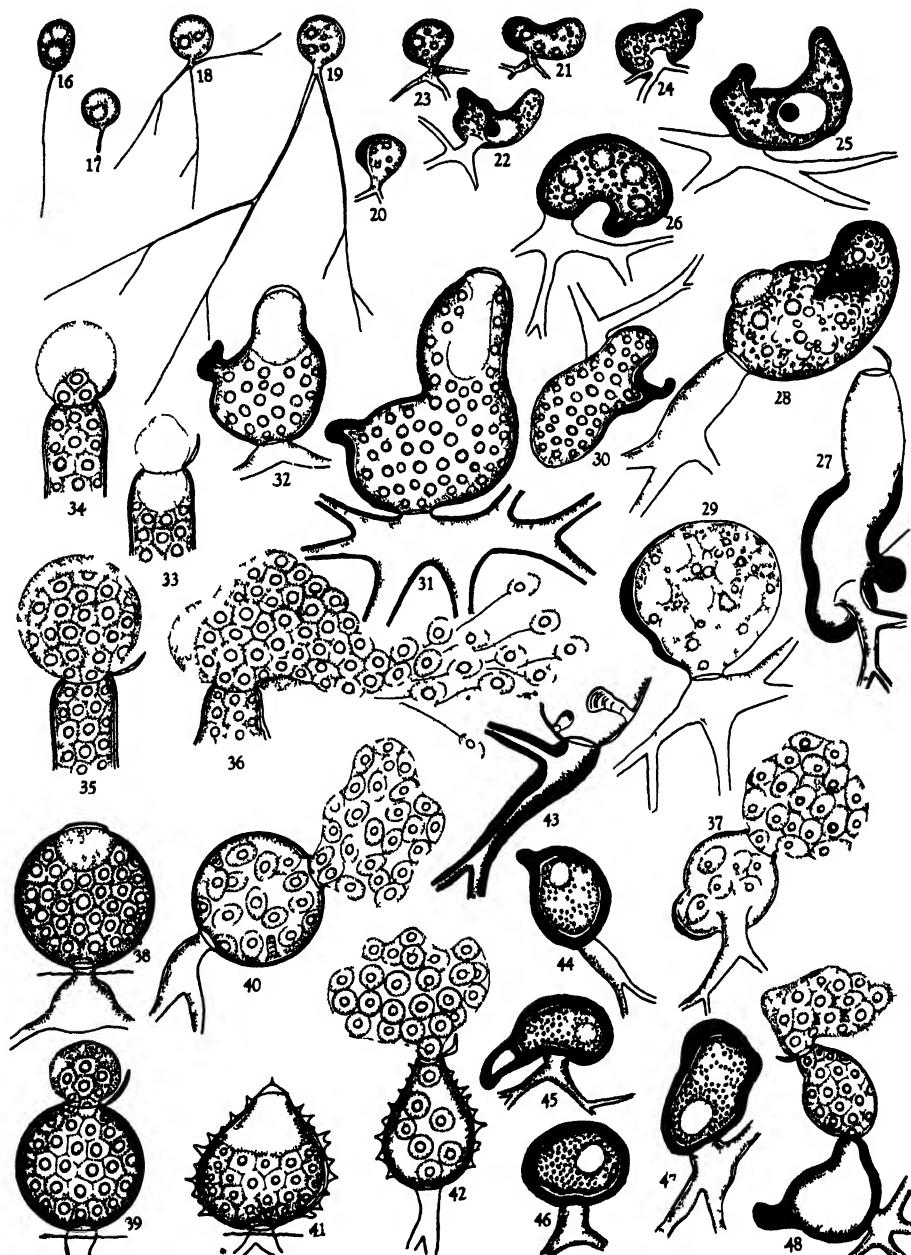
FIGS 16–37, 43–48 *Chytriomyces appendiculatus* FIGS 38–40 *C hyalinus* FIGS 41, 42 *C spinosus* FIG 16 Zoospore FIGS 17–19 Stages in zoospore germination in water FIG 20 Thickening of part of wall on incipient zoosporangium to form an appendage FIGS 21, 22 Asymmetrical, irregular incipient sporangia resulting from unilateral growth. FIGS 23–25 Incipient sporangia with uniformly thickened wall

(fig. 41). It may completely fill the apex of the sporangium (fig. 32) or extend down into the sporangium for a distance of 25 μ as a sac-shaped mass (fig. 31). This characteristic was particularly evident in the sporangia which developed in the Virginia, New Jersey, and Connecticut soil collections. However, in a collection of muck soil from Van Cortlandt Park in New York City, numerous sporangia were observed in which the matrix was very scarce or lacking (fig. 30) as in sporangia of *C. parasiticus*. Similar variations have also been noted in *C. aureus* and *C. hyalinus*.

When the sporangium dehisces, the operculum is pushed off and the hyaline matrix flows out (fig. 33) to form a globular mass at the exit orifice. The emerging zoospores push up into this mass (fig. 34) and expand it as in *C. hyalinus* (fig. 39), until it forms a thin enveloping layer. In the majority of sporangia from the Virginia, New Jersey, and Connecticut collections, this layer usually burst at one side, before most of the spores had emerged. The zoospores then flowed away in extended masses from the exit orifice (figs. 36, 48) and separated slowly. After a lapse of 2–3 minutes the individual spores began to jerk about and soon thereafter swam away. This behavior led the author to believe that the zoospores of *C. appendiculatus* do not swarm in a vesicle like those of *C. hyalinus* (fig. 40), *C. aureus*, *C. spinosus* (fig. 42), *C. parasiticus*, and *C. stellatus*. However, in sporangia collected in Van Cortlandt Park, New York City, the zoospores swarmed in a typical vesicle (fig. 37). Either the behavior of the zoospores shown in figures 36 and 48 is abnormal, or the behavior of the emerged zoospores varies to some degree in *C. appendiculatus*. On the other hand, it is possible that the collection from New York City may include a different strain or variety of this species. The active swarming period lasts from 20 to 75 minutes, after which the zoospores came to rest and became almost spherical. They germinate quite readily in the water surrounding the bits of chitin (fig. 17) and form extensive, branched rhizoids (figs. 18, 19) before increasing markedly in diameter.

The rhizoids arise from the base of the sporangium, and in large thalli

FIG. 26. Thick-walled, immature, dormant sporangium with granular and globular refractive content. FIG. 27. Later, "germinated" stage of a similar sporangium. FIG. 28. Irregular sporangium with a large single rhizoidal axis. FIG. 29. Vacuolate globular sporangium. FIG. 30. Mature, appendiculate sporangium lacking hyaline matrix at apex. FIG. 31. Mature sporangium with a sac-like mass of hyaline matrix extending down among incipient zoospores. FIG. 32. Mature sporangium with apex filled with matrix. FIGS. 33–35. Extrusion of matrix and its expansion by the emerging zoospores. FIG. 36. Rupture of matrical layer and dispersal of zoospores without formation of a vesicle. FIG. 37. Swarming of zoospores in a vesicle. FIG. 38. Hemispherical mass of slimy matrix under operculum. FIGS. 39–40. Extrusion of matrix and swarming of zoospores in a vesicle; note nodular plugs of wall material in latter figure. FIGS. 41–42. Matrix in sporangium and swarming of zoospores in a vesicle. FIG. 43. Thick-walled, brown rhizoidal axis; note nodular plugs of wall material in sporangium. FIGS 44–47. Variations in size and shape of resting spores. FIG. 48. Germination of resting spore.

they are unusually coarse and large in diameter. In a few exceptional thalli observed, the main axes of the rhizoids were up to 18 μ in diameter (fig. 28). The main axes branch obtusely and usually infrequently and do not bear very many small lateral branches. As a result, they often have the "stiff," abruptly tapering appearance that is characteristic of most species of *Rhizidium*. In old large thalli the rhizoidal wall may become greatly thickened, up to 5.5 μ, and turns brown in color (figs. 31, 43) 'like the wall of the sporangium. No clearly defined apophysis has been observed in *C. appendiculatus*, but the junction of the large rhizoids at the base of the sporangia often creates a large area which may resemble a digitate apophysis (figs. 29, 31).

Occasionally, clavate or irregular, nodular, plug-like ingrowths of the sporangium and rhizoidal wall occur (fig. 43). Sometimes, these resemble the tyloses of higher plants, and often show stratification, as if successive layers of wall material had been deposited at the apices. In other instances, they appear as extended, irregular and nodular thickenings of the inner surface of the wall. Similar plugs and thickenings have been observed in *C. aureus*, *C. hyalinus* (fig. 40), *C. stellatus*, and *Rhizophlyctic petersenii*. They appear to be formed only under certain conditions which are not yet clearly understood.

In connection with these nodular plugs or ingrowths, it may be noted that Haskins created *C. nodulatus* on the basis of the presence of such structures in the sporangia. Except for a very slight difference in zoospore size, which does not seem sufficiently great for specific distinction, and the presence of a larger number of nodular plugs, *C. nodulatus* appears to be identical to *C. hyalinus*. In view of the fact that such nodules have been found in *C. hyalinus* and other species, it seems doubtful that their presence is a specific, diagnostic character. Therefore, *C. nodulatus* may be identical to *C. hyalinus*, or a variety of it.

So far, resting spores' have been found only occasionally. They are usually appendiculate (figs. 44, 45, 48) and irregular in shape, although oval (fig. 46), almost spherical, and non-appendiculate ones occur also. Their wall is dark brown in color and varies from 2 to 5 μ in thickness. Sometimes it may be thicker over one portion of the spore, as shown in figure 47. The content of the spore is coarsely but evenly granular with a conspicuous clear area which has the appearance of a vacuole. In germinating, the resting spores function as prosporangia and give rise to a hyaline superficial, thin-walled, operculate zoosporangium (fig. 48).

CHYTRIOMYCES STELLATUS. A few resting spores and sporangia of this species were first observed on the skeleton of an unidentified insect collected in a swamp at São Carlos, Matto Grosso, Brazil. Because of the appearance

and structure of the resting spores, it was regarded at first as a species of *Asterophlyctis,* but when the sporangia proved to be operculate, it was obvious that they belonged to a new species of *Chytriomyces.* Subsequently this species was isolated from water and muck soil collected in New York and Connecticut, which indicates that it may be widely distributed in nature. The life cycle and structure of *C. stellatus* will be illustrated fully in a subsequent paper dealing with the cytology of the genus *Chytriomyces,* and the present description will be confined to the specific distinguishing characteristics.

The mature sporangia are predominantly oval, subspherical, broadly pyriform, hyaline, and usually smooth with one to three apical or sub-apical exit papillae. However, sporangia with 1–5 short, solid, hyaline, triangular pegs or spines are not uncommon, and the exit papillae may be replaced frequently by 1–3 short or long, broad exit tubes. Anatropous, slightly reniform and angular sporangia also occur in this species. As in other members of *Chytriomyces* a large amount of homogeneous slimy material is usually present beneath the operculum, and in some sporangia it may fill the exit tube completely. The primary nucleus of the sporangium is quite large and stands out clearly in living material a clear globular body with a dense nucleole. It keeps pace with the growth and increase in size of the sporangium and does not divide until the latter has attained mature size.

The subsporangial swelling or apophysis is usually large and conspicuous and may sometimes attain a diameter of 18 μ. It varies from spherical to oval, broadly fusiform and slightly angular in shape, with the rhizoids arising from its base or at several points on its surface. The rhizoids are not very extensive but branch frequently, so that a close mat of branches is usually formed in the substratum. Sometimes a central unbranched axis, 8–20 μ long, is formed beneath the apophysis, and from the base of this axis arises a dense tuft of rhizoids.

The dehiscence of the sporangium, exudation of the slimy, hyaline matrix, formation of a vesicle, and the swarming of the zoospores in the vesicle are similar to those described for other species of *Chytriomyces* and need not be discussed again.

The resting spores are hyaline, apophysate, predominantly globular, and stellate in shape with from one to several large conspicuous refractive globules. The pegs or spines on the wall may be up to 6 μ in height in exceptional cases, blunt or sharply pointed, and sometimes bifurcate. Occasionally, they are reduced to warts or mere undulations on the wall, so that the spores appear to be verrucose. Smooth spores are rare and exceptional and possibly abnormal. The nucleus of the developing spore is clearly visible in living material and may be observed until quite late, when it is dis-

placed towards the periphery by the large refractive globules. Like other species of *Chytriomyces,* the resting spores function as prosporangia in germinating and give rise to superficial, hyaline, thin-walled, operculate zoosporongia.

SUMMARY

Three new species of *Chytriomyces* have been isolated from fresh water and muck soil from the eastern part of the United States and the Amazon Valley in Brazil. *Chytriomyces parasiticus* parasitizes *Aphanomyces laevis* and causes marked local hypertrophy and excessive branching of the mycelium and filamentous sporangia. It is characterized primarily by minute zoospores. The other two species are saprophytes on chitinous substrata. *Chytriomyces appendiculatus* is distinguishable by large, appendiculate, irregular and variously-shaped sporangia, very coarse rhizoids, and thick-walled, brown, appendiculate resting spores. *Chytriomyces stellatus,* on the other hand, develops hyaline stellate resting spores and conspicuously apophysate sporangia, and may be distinguished chiefly by these characteristics.

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TORREYA

PROCEEDINGS OF THE CLUB

The following two abstracts were received too late to be published in the last issue of the BULLETIN with the minutes of the meetings at which the talks were given.

At the meeting of January 15, 1947, Dr. Helen Purdy Beale spoke on ''Some Properties of the Tobacco Mosaic Virus.''

The relationship between the various forms of tobacco mosaic virus was discussed. Using wooden models constructed to scale, the relative size of the smallest particle of virus, as it appears in electron micrographs, was compared with the paracrystals and hexagonal plate crystals. Lantern slides showed the transformation of the plate crystals within the infected host cells to the needle-like paracrystalline form.

An outline was then given of the work concerned with the antigenic property of tobacco virus protein. When virus was injected into animals, it stimulated the production of specific antibody in the serum of the animal. This was shown to be a most useful reaction in determining relationships between viruses, the detection of symptomless carriers, and also in estimating the total concentration of virus protein in a given preparation. Only active virus could be detected by plant inoculation, but both active and inactive virus protein, provided it had not become denatured, reacted with serum antibody. The current theory of antibody production, as outlined by F. M. Burnet in his monograph, was presented.

In conclusion, the interference reaction between tobacco mosaic virus and a related strain was discussed. Although the latter, known as the rib-grass strain, was incapable of infecting bean, when this strain was mixed with the common field strain of tobacco mosaic virus, prior to inoculation of bean, the number of infective local lesions which the field strain alone was capable of inducing was reduced. This phenomenon was compared to similar ones in the field of bacteriophage and animal viruses.

At the meeting of February 5, 1947, Dr. L. M. Black spoke on ''Plant Tumors Induced by Viruses.''

Among the overgrowths produced in plants by virus infection those occurring in Fiji-disease and in wound-tumor disease are distinct galls or tumors. Infections with wound-tumor virus have been obtained in the greenhouse on 43 species of plants in 20 families. The most consistent symptoms of the disease are irregular enlargement of the veins of leaves, and tumors on roots. However, these symptoms may be inconspicuous on some suspects and other symptoms may occur. Tissue culture studies have demonstrated that the tumors are capable of indefinite growth as tumor tissue and that they retain the virus. Wounds start tumors in infected plants. The virus is not transmitted to healthy plants by rubbing their leaves with juice from diseased plants. It is transmitted by the leafhoppers *Agallia constricta* Van Duzee, *Agallia quadripunctata* (Provancher) and *Agalliopsis novella* (Say). Hereditary differences in tumor response have been demonstrated in sweet clover. Many similarities exist between wound-tumor disease and animal virus tumors such as mammary cancer of the mouse.

Minutes of the Meeting of March 4, 1947. The regular evening meeting of the Torrey Botanical Club was opened by Dr. Shull at 8:00 p.m. at Columbia University with 70 members and friends present. The minutes of the previous meeting were not read.

No business was transacted at this meeting and Dr. Shull immediately introduced the speaker of the evening, Dr. David R. Goddard of the University of Pennsylvania who spoke on ''The Cytochrome System and Peroxidase in Plant Respiration.''

The meeting was adjourned at 9: 15 p.m. after a lengthy discussion, and refreshments were served by members of the staff of the Columbia Botany Department.

Respectfully submitted,

Libero Ajello,

Recording Secretary

Minutes of the Meeting of March 19, 1947. The regular afternoon meeting of the Torrey Botanical Club was called to order by Dr. Shull at 4: 00 p.m. in Larkin Hall, Fordham University. Fifty members and friends were present. The minutes of the meetings of February 19 and March 4 were approved as read. Three associate members and 2 annual members were elected into the Club.

Following this brief business meeting, Dr. Shull introduced Dr. C. A. Berger, who presented a very full report on ''Cyto-botanical research at Fordham University.'' The talk, abstracted below, was illustrated with vivid diagrams and excellent photomicrographs of various cytological preparations. A number of slides were set up under microscopes for examination by interested members.

Cytological research at Fordham has centered around the phenomenon of Polyploidy as found in certain tissues or cells of diploid plants. Three aspects of this subject have been investigated. They are (1) polyploidy as a factor in the normal development of diploid plants; (2) artificially induced polyploidy; (3) the mechanism of origin of polyploid cells in normal and treated material.

Under the heading of naturally occurring polyploidy, polysomaty was studied in *Spinacia, Chenopodium, Atriplex* and *Kochia;* certain cortical cells of the root of *Mimosa* and of the shoot of *Allium* were found to become tetraploid and to divide once as tetraploids at a definite stage in the development of the seedling; the formation of various types of multinucleate and polyploid cells was investigated in the tapetal cells of *Spinacia, Coreopsis, Lilium, Symplocarpus, Magnolia* and *Rhoeo.*

Colchicine, acenaphthene, sulfanilamide, chloral hydrate, veratrine and podophyllin were used to induce polyploidy. Alpha-naphthalene-acetic acid was found to stimulate mitosis in differentiated cells which had already become polyploid but not to induce the polyploid condition. Combined treatment with naphthalene-acetic acid followed by colchicine was found to result in a considerable increase in the frequency of polyploid cells.

In many of the above studies it was possible to determine the mechanism by which the polyploid condition arose. In general, induced polyploidy results from some failure of the mitotic process while naturally occurring polyploidy results from a double reproduction of chromosomes in the resting nucleus or less commonly from endomitosis.

Several problems not connected with polyploidy are also being investigated. Some of these are: chromosome spirallization in *Trillium;* mitosis in yeast and a cytological analysis of Hybrid Petunias from the experimental gardens of Dr A. B. Stout of the New York Botanical Garden. Approximately 180 segregates from diploid and induced-tetraploid crosses have been analyzed and numbers ranging from the diploid 14 to the hexaploid 42 have been determined.

The meeting was adjourned at 5: 00 p.m. Refreshments had been served prior to the meeting.

Respectfully submitted,

Libero Ajello,

Recording Secretary

News Notes

The Beckman Herbarium, possibly the oldest herbarium in the United States, has been given to the California Academy of Sciences by Mrs. N. Floy Bracelin of Berkeley, to whom it had previously been given by Miss Sara Beckman, a descendant of the collector. According to Dr. Robert C. Miller, the Director of the Academy, this herbarium

is a book of pressed plants prepared by Anders Beckman and completed in 1752. It is thought that Beckman studied under Linnaeus. In his herbarium he uses the system of nomenclature published by Linnaeus in "Flora Suecica" in 1745. After nearly 200 years, many of the pressed plants are still in good condition and easily recognizable. The book apparently has been much used, and some specimens are missing, being identifiable chiefly by the imprints left on the pages.

The Library of Congress has announced a new cataloging service for libraries, called the *Cumulative Catalog of Library of Congress Printed Cards*. It is a monthly bulletin of about 125 pages, each page containing in reduced facsimile the information from about 35 catalog cards. Quarterly and annual cumulative bulletins are also planned. The subscription price of the *Cumulative Catalog* is $100 a year.

Since 1901 the Library of Congress has sold sets of its catalog cards, and later the card depository system was established by which a library, agreeing to maintain the cards available to the public in alphabetical order, received a set of cards on deposit free of charge. Both of these systems have proved rather costly for the libraries. In 1942 the Association of Research Libraries undertook the reprinting of the entire catalog in book form. This has been completed and fills 167 volumes under the title: *A Catalog of Books Represented by Library of Congress Printed Cards, through July 31, 1942. A Supplement, August 1, 1942 to December 31, 1946* is being prepared, and will probably fill 30 volumes. These catalogs with the continuing monthly bulletins will cover all of the Library of Congress catalog cards, and will be much cheaper and less bulky than the cards themselves. It is thought that these books will make the reference material of the Library of Congress catalog cards available to many more libraries than was possible heretofore.

The Southeastern Chapter of the Michigan Association for Native Plant Protection announces that the *Second Michigan Salon of Native Wildflower Photography* will be exhibited in March, 1948, Entry forms will be available in October, 1947, giving the closing date for entries, listing judges and showing exhibition dates. The Salon will be hung at the Flower Show of 1948 in Detroit, Michigan. There will be two divisions, one for monochrome and color prints and another for slides. Some suggested subjects are: Wildflowers—foliage & fruit; Native shrubs and trees—flowers and fruit; Native grasses, sedges, rushes, etc.; In situ or still life. The Chairman of the Salon Committee is Mr. Roger E. Richard, 1832 N. Gulley Rd., Dearborn, Michigan.

ABBREVIATIONS OF PERIODICALS

cited in the Index to American Botanical Literature[1]

compiled by

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This list was prepared because of the difficulty of attaining uniformity in citing periodicals in the *Index to American Botanical Literature* and *The Taxonomic Index*. Even a single compiler has some trouble in this respect; and this is multiplied by collaboration. The list having been made, it was felt that it might be a convenience to others having botanical literature to cite. In particular, contributors to the BULLETIN are asked to adopt these abbreviations in their citations. Furthermore, it may assist users of the *Index* who attempt to find the less familiar serials to have the full titles and other data provided below.

The serials here represented are those which have been cited in recent issues of the *Index*. While it is fairly complete for American botanical periodicals, the apparently haphazard inclusion of certain European serials and the omission of others can be understood only by reference to the aim of the *Index*—"to include all current botanical literature written by Americans, published in America, or based upon American material."

The aim of abbreviation is the utmost brevity consistent with clarity. Well known words are sufficiently represented by their first syllable, with or without an additional letter; as "Am.," "Agr.," "Jour." Two or more syllables may be necessary for distinctiveness, as "Bryol.," "Argent.," "Philos." (to distinguish it from Philadelphia, Philippine). Personal names, and titles consisting of one word are not abbreviated. It is frequently necessary, for the sake of precision, to add words (in brackets) not taken from the title; two recent periodicals have been named "Flora," and such titles as "Anales del Instituto de Biología" may be ambiguous without mention of the place of origin.

Each abbreviated title is followed by the complete title as it appears on the current cover or title-page of the serial. (When these differ, the latter is taken as correct.) Variations in the title are shown by parentheses. Other information taken from the title-page but not properly to be regarded as part of the title is appended in brackets.

In the *Index* the series, volume, page, etc. of the serial are indicated in accord with the rules adopted at the Madison Botanical Congress of 1893 (see Bull. Torrey Club 22: 130–132. 27 Mr 1895). In brief, the method is as follows. The number of the *series*, if any, is in Roman numerals. The *volume* is designated by arabic numerals in bold face, followed by a colon. (When the volumes are not numbered, years are used instead; e.g. Kew Bull. 1941: 218–227. 9 Mr 1942.) If *parts* are independently paged, they are indicated by a superscript number attached to the number of the volume; otherwise they are not specified. The first and last *pages* of the article are in arabic numerals connected by a dash (if more than two; two consecutive pages are separated by a comma). Numbers of *figures* and *plates* are in italic and arabic, preceded by *f.* and *pl.* respectively. The date of publication is abbreviated as recommended by the Library Bureau, brackets being used to distinguish between actual dates and inaccurate dates.

The abbreviations listed below are, with a few exceptions, also those used in North American Flora.

[1] A limited number of separate copies of this list are available from H. W. Rickett (The New York Botanical Garden, New York 58, N. Y.) at a cost of 15 cents each.

Acadian Nat.—Acadian Naturalist. Bulletin of the Natural History Society of New Brunswick.

Acta Bot. Fenn.—Acta Botanica Fennica. [Societas pro Fauna et Flora Fennica.]

Acta Hort. Gothob.—Acta Horti Gothoburgensis. Meddelanden från Göteborgs Botaniska Trädgård.

Acta Phytogeogr. Suec.—Acta Phytogeographica Suecica. [Svenska Växtgeo-grafiska Sällskapet.]

Acta Soc. Faun. Fl. Fenn.—Acta Societatis pro Fauna et Flora Fennica.

Addisonia—Addisonia. [The New York Botanical Garden.]

Agr. Hist.—Agricultural History. [Washington.]

Agr. Téc. [Santiago]—Agricultura Técnica. [Santiago de Chile.]

Am. Fern Jour.—American Fern Journal. [The American Fern Society.]

Am. Fruit Grower—American Fruit Grower.

Am. Jour. Bot.—American Journal of Botany. [The Botanical Society of America.]

Am. Jour. Sci.—American Journal of Science. [New Haven.]

Am. Midl. Nat.—The American Midland Naturalist. [Notre Dame University.]

Am. Nat.—The American Naturalist. [Lancaster.]

Am. Orchid Soc. Bull.—American Orchid Society Bulletin.

An. Acad. Brasil. Ci.—Anais da Academia Brasileira de Ciências. [Rio de Janeiro. Vols. 1–12 (1929–1940) as Annaes da Academia Brasileira de Sciencias.]

Anal. Esc. Nac. Ci. Biol. [Mexico]—Anales de la Escuela Nacional de Ciencias Biológicas. [Mexico.]

Anal. Inst. Biol. [Mexico]—Anales del Instituto de Biología de la Universidad Nacional de México.

Anal. Mus. Argent.—Anales del Museo Argentino de Ciencias Naturales. [Buenos Aires.]

Anal. Soc. Ci. Argent.—Anales de la Sociedad Científica Argentina. [Buenos Aires.]

Ann. ACFAS—Annales de l'ACFAS. [Association Canadienne Française pour l'Avancement des Sciences.]

Ann. Bot.—Annals of Botany. [Oxford.]

Ann. Cryptog. Phytopath.—Annales Cryptogamici et Phytopathologici. [Includ-ing Annales Bryologici.]

Ann. Mo. Bot. Gard.—Annals of the Missouri Botanical Garden.

Ann. N. Y. Acad.—Annals of the New York Academy of Sciences.

Appalachia—Appalachia. [The Appalachian Mountain Club.]

Arb. Bull.—Arboretum Bulletin. [Seattle.]

Arch. Inst. Bot. [Liége]—Archives de l'Institut de Botanique de l'Université de Liége.

Ark. Bot.—Arkiv för Botanik. [K. Svenska Vetenskapsakademien.]

Arq. Bot. Est. S. Paulo—Arquivos de Botânica do Estado de São Paulo.

Arq. Inst. Biol. [S. Paulo]—Arquivos do Instituto Biologico. Departamento da Defesa Sanitária da Agricultura. [São Paulo.]

Arq. Mus. Nac. [Rio de Janeiro]—Arquivos do Museu Nacional. [Rio de Janeiro.]

Arq. Serv. Flor. [Rio de Janeiro]—Arquivos do Serviço Florestal. [Ministério da Agricultura. Rio de Janeiro.]

Atti Ist. Bot. Pavia—Atti dell' Istituto Botanico "Giovanni Briosi" e Labora-torio Crittogamico Italiano della R. Università di Pavia.

Bartonia—Bartonia. Journal of the Philadelphia Botanical Club.

Beih. Bot. Centr.—Beihefte zum Botanischen Centralblatt.

Ber. Deuts. Bot. Ges.—Berichte der Deutschen Botanischen Gesellschaft.

Ber. Schweiz. Bot. Ges.—Berichte der Schweizerischen Botanischen Gesellschaft. [Bulletin de la Société Botanique Suisse.]

Biologist—The Biologist. [The Phi Sigma Society.]

Bishop Mus. Bull.—Bernice P. Bishop Museum Bulletin. [Honolulu.]

Bishop Mus. Spec. Publ.—Bernice P. Bishop Museum Special Publication. [Honolulu.]

Blumea—Blumea. [Leiden.]

Bol. Fitossan. [Rio de Janeiro]—Boletim Fitossanitário. [Divisão de Defesa Sanitária Vegetal. Rio de Janeiro.]

Bol. Inst. Bot. Univ. Cent. [Quito]—Boletín del Instituto Botánico de la Universidad Central. [Quito.]

Bol. Mus. Hist. Nat. [Lima]—Boletín del Museo de Historia Natural ''Javier Prado.'' [Lima.]

Bol. Mus. Nac. [Rio de Janeiro]—Boletim do Museu Nacional. [Rio de Janeiro.]

Bol. Soc. Argent. Bot.—Boletín de la Sociedad Argentina de Botánica.

Bol. Soc. Brot.—Boletim da Sociedade Broteriana. [Instituto Botânico da Universidade de Coimbra.]

Bol. Soc. Geogr. Lima—Boletín de la Sociedad Geográfica de Lima.

Bol. Soc. Venez. Ci. Nat.—Boletín de la Sociedad Venezolana de Ciencias Naturales.

Bol. Téc. Dep. Genet. Fitotéc. [Santiago]—Boletín técnico. Ministerio de Agricultura. Departamento de Genética Fitotécnica. [Santiago de Chile.]

Bol. Téc. Inst. Agron. Norte [Belém]—Boletim técnico do Instituto Agronômico do Norte. [Belém.]

Bol. Téc. Inst. Cacau Bahia—Boletim técnico. Instituto de Cacau da Bahia.

Bot. Arch.—Botanisches Archiv. [Leipzig.]

Bot. Gaz.—The Botanical Gazette. [The University of Chicago.]

Bot. Mag.— Curtis's Botanical Magazine. [The Royal Horticultural Society, London.]

Bot. Mus. Leafl.— Botanical Museum Leaflets. [Harvard University.]

Bot. Not.—Botaniska Notiser. [Lunds Botaniska Förening.]

Bot. Rev.—The Botanical Review. [New York.]

Bot. Tidsskr.—Botanisk Tidsskrift. [Dansk Botanisk Forening.]

Bothalia—Bothalia. [Pretoria.]

Brit. Mycol. Soc. Trans.—The British Mycological Society. Transactions.

Brittonia—Brittonia. [The New York Botanical Garden.]

Bryologist—The Bryologist. Journal of the Sullivant Moss Society.

Bull. Am. Rock Gard. Soc.—Bulletin of the American Rock Garden Society. Including Saxiflora.

Bull. Jard. Bot. [Bruxelles]—Bulletin du Jardin Botanique de l'État. [Bruxelles.]

Bull. Mus. Hist. Nat. [Paris]—Bulletin du Muséum National d'Histoire Naturelle. [Paris.]

Bull. Scripps Inst. Oceanogr.—Bulletin of the Scripps Institution of Oceanography (of the University of California. La Jolla, California). Technical Series.

Bull. So. Calif. Acad.—Bulletin of the Southern California Academy of Sciences.

Bull. Soc. Bot. Fr.—Bulletin de la Société Botanique de France.

Bull. Soc. Bot. Genève—Bulletin de la Société Botanique de Genève.

Bull. Soc. Hist. Nat. Toulouse—Bulletin de la Société d'Histoire Naturelle de Toulouse.

Bull. Torrey Club—Bulletin of the Torrey Botanical Club and Torreya.

Butler Univ. Bot. Stud.—Butler University Botanical Studies.

Cactus & Succ. Jour.—Cactus and Succulent Journal of the Cactus and Succulent Society of America.

Caldasia—Caldasia. Boletín del Instituto de Ciencias Naturales de la Universidad Nacional de Colombia—Bogotá.

Canad. Field-Nat.—The Canadian Field-Naturalist.

Canad. Jour. Res. C.—Canadian Journal of Research. Section C. Botanical Sciences.

Candollea—Candollea. Organe du Conservatoire et du Jardin Botaniques de la Ville de Genève.

Carib. Forest.—The Caribbean Forester. [Puerto Rico.]

Carn. Inst. Wash. Year Book—Carnegie Institution of Washington. Year Book.

Castanea—Castanea. The Journal of the Southern Appalachian Botanical Club.

Cellule—La Cellule. Recueil de cytologie et d'histologie. [Louvain.]

Ceres—Revista Ceres. [Viçosa, Minas Gerais.]

Chron. Bot.—Chronica Botanica. [Waltham, Massachusetts.]

Circ. U. S. Dep. Agr.—Circular. United States Department of Agriculture.

Compt. Rend. Acad. U.R.S.S. II.—Comptes Rendues (Doklady) de l'Académie des Sciences de l'URSS. Nouvelle série.

Contr. Boyce Thompson Inst.—Contributions from Boyce Thompson Institute. [Yonkers, New York.]

Contr. Dudley Herb.—Contributions from the Dudley Herbarium (of Stanford University). [Offset.]

Contr. Gray Herb.—Contributions of the Gray Herbarium of Harvard University.

Contr. Inst. Bot. Univ. Montréal—Contributions de l'Institut Botanique de l'Université de Montréal.

Contr. Inst. Oka—Contributions de l'Institut d'Oka (Université de Montréal).

Contr. Ocas. Mus. Hist. Nat. Col. ''De La Salle'' [Havana.]—Contribuciones Ocasionales del Museo de Historia Natural del Colegio ''De La Salle.'' [Havana.]

Contr. U. S. Nat. Herb.—Contributions from the United States National Herbarium.

Contr. Univ. Mich. Herb.—Contributions from the University of Michigan Herbarium.

Cranbrook Inst. Bull.—Cranbrook Institute of Science. Bulletin.

Dansk Bot. Ark.—Dansk Botanisk Arkiv (Res Botanicae Danicae). [Dansk Botanisk Forening.]

Danske Vid. Selsk. Biol. Meddel.—Kgl. Danske Videnskabernes Selskab. Biologiske Meddelelser.

Darwiniana—Darwiniana. Revista del Instituto de Botánica Darwinion. [Academia Nacional de Ciencias Exactas, Físicas y Naturales de Buenos Aires.]

Des. Pl. Life—Desert Plant Life. [American Succulent Societies.]

Ecol. Monogr.—Ecological Monographs. [The Ecological Society of America.]

Ecology—Ecology. [The Ecological Society of America.]

Econ. Bot.—Economic Botany. [New York.]
Evolution—Evolution. International Journal of Organic Evolution. [The Society for the Study of Evolution.]

Farlowia—Farlowia. A journal of cryptogamic botany. [Farlow Library and Herbarium of Harvard University.]
Fauna & Flora [Uppsala]—Fauna och Flora. Populär Tidskrift för Biologi. [Uppsala.]
Field & Lab.—Field and Laboratory. Contributions from the Science Departments of Southern Methodist University.
Fieldiana Bot.—Fieldiana: Botany. [Formerly "Field Museum of Natural History. Publications. Botany." The Chicago Natural History Museum.]
Fla. Geol. Surv. Rep.—Florida Geological Survey. Annual Report.
Flora—Flora oder Allgemeine Botanische Zeitung. [Jena.]
Flora [Quito]—Flora. Revista de Botánica y Farmacognosia. Organo oficial del Instituto Botánico de la Universidad Central. [Quito.]

Genetica—Genetica. Nederlandsch Tijdschrift voor Ervelijkheids- en Afstemmingsleer.
Genetics—Genetics. [The Brooklyn Botanic Garden.]
Gent. Herb.—Gentes Herbarum. (Occasional papers on the kinds of plants.) [The Bailey Hortorium, Cornell University.]
Geogr. Rev.—The Geographical Review. [The American Geographical Society.]
Great Basin Nat.—The Great Basin Naturalist. [Provo, Utah.]

Hereditas—Hereditas. Genetiskt Arkiv. [Mendelska Sällskapet i Lund.]
Holmbergia—Holmbergia. Revista del Centro de Estudiantes del Doctorado en Ciencias Naturales. [Buenos Aires.]
Hook. Ic.—Hooker's Icones Plantarum. [Kew.]

Ill. Biol. Monogr.—Illinois Biological Monographs.
Inst. Agron S. Paulo Bol. Téc.—Instituto Agronômico do Estado [de São Paulo], em Campinas. Boletim técnico.
Iowa St. Coll. Jour. Sci.—Iowa State College Journal of Science.

Jour. Agr. Res.—Journal of Agricultural Research. [Washington.]
Jour. Agr. Sci.—The Journal of Agricultural Science. [Cambridge.]
Jour. Agr. Univ. Puerto Rico—The Journal of Agriculture of the University of Puerto Rico.
Jour. Am. Soc. Agron.—Journal of the American Society of Agronomy.
Jour. Arnold Arb.—Journal of the Arnold Arboretum. [Harvard University.]
Jour. Bot.—The Journal of Botany, British and foreign.
Jour. Calif. Hort. Soc.—Journal of the California Horticultural Society.
Jour. Elisha Mitchell Soc.—Journal of the Elisha Mitchell Scientific Society.
Jour. Forest.—Journal of Forestry. [The Society of American Foresters.]
Jour. Gen. Physiol.—The Journal of General Physiology. [The Rockefeller Institute for Medical Research.]
Jour. Hered.—The Journal of Heredity. [The American Genetic Association.]
Jour. Linn. Soc.—The Journal of the Linnean Society of London. Botany.
Jour. N. Y. Bot. Gard.—Journal of the New York Botanical Garden.
Jour. Sci. Lab. Denison Univ.—Journal of the Scientific Laboratories of Denison University.

Jour. Tenn. Acad.—Journal of the Tennessee Academy of Science.
Jour. Wash. Acad.—Journal of the Washington [D.C.] Academy of Sciences.

Kew Bull.—Royal Botanic Gardens, Kew. Bulletin of Miscellaneous Information.
Kolon. Inst. Amsterdam Meded.—Vereeniging Koloniaal Instituut te Amsterdam. Mededeeling.

Leafl. West. Bot.—Leaflets of Western Botany.
Lilloa—Lilloa. Revista de Botánica. [Universidad Nacional de Tucumán. Instituto "Miguel Lillo."]
Lloydia—Lloydia. A quarterly journal of biological science. [Lloyd Library and Museum.]
Lunds Univ. Årssk. II. Sect. 2.—Lunds Universitets Årsskrift. N[ya] F[öljd]. Avd[elning] 2.

Madroño—Madroño, a West American Journal of Botany.
Meded. Bot. Mus. Utrecht—Mededeelingen van het Botanisch Museum en Herbarium van de Rijksuniversiteit te Utrecht.
Mem. Gray Herb.—Memoirs of the Gray Herbarium of Harvard University.
Mem. Mus. Entre Rios [Argentina]—Memorias del Museo de Entre Rios. República Argentina. [Formerly entitled "Las Memorias del Museo de Paraná."]
Mem. Soc. Cub. Hist. Nat.—Memorias de la Sociedad Cubana de Historia Natural "Felipe Poey."
Mem. Soc. Faun. Fl. Fenn.—Memoranda Societatis pro Fauna et Flora Fennica.
Mém. Soc. Vaud. Sci. Nat.—Mémoires de la Société Vaudoise des Sciences Naturelles. [Lausanne.]
Mem. Torrey Club—Memoirs of the Torrey Botanical Club.
Mem. & Revista Acad. Nac. [Mexico]—Memorias y Revista de la Academia Nacional de Ciencias ("Antonio Alzate"). [Mexico.]
Mo. Bot. Gard. Bull.—Missouri Botanical Garden Bulletin.
Mycologia—Mycologia. [The New York Botanical Garden.]

N. Am. Flora—North American Flora. [The New York Botanical Garden.]
Nat. Canad.—Le Naturaliste Canadien.
Nat. Hist. Bull. Hawaii Nat. Park.—Natural History Bulletin. Hawaii National Park. [Mimeographed.]
Nat. Hort. Mag.—The National Horticultural Magazine. Journal of the American Horticultural Society.
Nature—Nature. [London.]
New Phytol.—The New Phytologist. [Cambridge, England.]
Northw. Sci.—Northwest Science. [The Northwest Scientific Association. Cheney, Washington.]
Not. Nat —Notulae Naturae of the Academy of Natural Sciences of Philadelphia.
Not. Syst. [Leningrad]—Notulae Systematicae ex Herbario Horti Botanici U.S.S.R. Botanicheskie Materialy.
Not. Syst. [Paris]—Muséum national d'histoire naturelle. Notulae Systematicae. [Paris.]
Notas Mus. La Plata Bot.—Notas del Museo de la Plata. Botánica.

Occ. Pap. Bishop Mus.—Occasional Papers of the Bernice Pauahi Bishop Museum of Polynesian Ethnology and Natural History. [Honolulu.]
Occ. Pap. Fairchild Trop. Gard.—Occasional Paper. Fairchild Tropical Garden.
Ohio Jour. Sci.—The Ohio Journal of Science.

Orquídea [Mexico]—Orquídea. Organo oficial de la Sociedad Mexicana ''Amigos de las Orquídeas.''

Orquídea [Rio de Janeiro]—Orquídea. | Rio de Janeiro.]

Oxford Forest. Mem.—Oxford Forestry Memoirs.

Pacif. Sci.—Pacific Science; a quarterly devoted to the biological and physical sciences of the Pacific region. [University of Hawaii.]

Pap. Mich. Acad.—Papers of the Michigan Academy of Science, Arts and Letters.

Pap. Tortugas Lab.—Papers from the Tortugas Laboratory of the Carnegie Institution of Washington.

Philipp. Jour. Sci.—The Philippine Journal of Science.

Physis—Physis. Revista de la Sociedad Argentina de Ciencias Naturales.

Phytologia—Phytologia. | New York. Lithoprinted.]

Phytopathology—Phytopathology. [The American Phytopathological Society.]

Plant Physiol.—Plant Physiology. [The American Society of Plant Physiologists.]

Proc. Acad. Phila.—Proceedings of the Academy of Natural Sciences of Philadelphia.

Proc. Am. Acad.—Proceedings of the American Academy of Arts and Sciences.

Proc. Am. Philos. Soc.—Proceedings of the American Philosophical Society held at Philadelphia for promoting useful knowledge.

Proc. Am. Soc. Hort. Sci.—Proceedings of the American Society for Horticultural Science.

Proc. Ark. Acad.—Proceedings of the Arkansas Academy of Science.

Proc. Biol. Soc. Wash.—Proceedings of the Biological Society of Washington [D. C.].

Proc. Calif. Acad.—Proceedings of the California Academy of Sciences.

Proc. Ind. Acad.—Proceedings of the Indiana Academy of Science.

Proc. Iowa Acad.—Proceedings of the Iowa Academy of Science.

Proc. La. Acad.—The Proceedings of the Louisiana Academy of Sciences.

Proc. Linn. Soc.—Proceedings of the Linnean Society of London.

Proc. Mont. Acad.—Proceedings of the Montana Academy of Sciences.

Proc. Nat. Acad.—Proceedings of the National Academy of Sciences of the United States of America.

Proc. Okla. Acad.—Proceedings of the Oklahoma Academy of Science.

Proc. Pa. Acad.—Proceedings of the Pennsylvania Academy of Science.

Proc. Roy. Dublin Soc.—The Scientific Proceedings of the Royal Dublin Society. New Series.

Proc. U. S. Nat. Mus.—Proceedings of the United States National Museum.

Proc. W. Va. Acad.—West Virginia University. Bulletin. Proceedings of the West Virginia Academy of Science.

Proc. & Trans. Nova Scotian Inst.—The Proceedings and Transactions of the Nova Scotian Institute of Science.

Proc. & Trans. Roy. Soc. Canada—Proceedings and Transactions of the Royal Society of Canada. (Mémoires et Comptes Rendus de la Société Royale du Canada.)

Quart. Jour. Fla. Acad.—Quarterly Journal of the Florida Academy of Sciences. [Formerly Proceedings.]

Quart. Rev. Biol.—The Quarterly Review of Biology. [Baltimore.]

Rec. Trav. Bot. Néerl.—Recueil des travaux botaniques néerlandais. [Société Botanique Royale Néerlandaise.]

Rep. Mich. Acad.—Annual Report of the Michigan Academy of Science, Arts and Letters.

Rep. Smithson. Inst.—Annual Report of the Board of Regents of the Smithsonian Institution.

Res. Stud. St. Coll. Wash.—Research Studies of the State College of Washington.

Revista Acad. Colomb.—Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales.

Revista Argent. Agron.—Revista Argentina de Agronomía.

Revista Chil. Hist. Nat.—Revista Chilena de Historia Natural.

Revista Fac. Agron. [Medellin]—Revista. Facultad Nacional de Agronomía. [Medellin.]

Revista Invest. Agr. [Buenos Aires]—Revista de Investigaciones Agricolas. Organo oficial de la Dirección General de Investigaciones del Ministerio de Agricultura de la Nación. [Buenos Aires.]

Revista Mus. La Plata Bot.—Revista del Museo de La Plata. Sección Botánica.

Revista Soc. Cub. Bot.—Revista de la Sociedad Cubana de Botánica. Organo oficial del Jardín Botánico de la Universidad de la Habana.

Revista Soc. Mex. Hist. Nat.—Revista de la Sociedad Mexicana de Historia Natural.

Revista Sudam. Bot.—Revista Sudamericana de Botánica. [Montevideo.]

Revista Univ. Catól. Chile—Revista Universitaria. Anales de la Academia Chilena de Ciencias Naturales. Universidad Católica de Chile.

Revue Bot. Appl. Agr. Trop.—Revue de Botanique Appliquée et d'Agriculture Tropicale.

Revue Bryol. Lichénol.—Revue Bryologique et Lichénologique. [Revue Bryologique, Series II.]

Revue d'Oka—Revue d'Oka. [Institut d'Oka, Université de Montréal.]

Rhodora—Rhodora. Journal of the New England Botanical Club.

Rodriguésia—Rodriguésia. Revista do Jardim Botânico. Serviço Florestal. [Rio de Janeiro.]

Sargentia—Sargentia. A continuation of the Contributions from the Arnold Arboretum of Harvard University.

Sci. Monthly—The Scientific Monthly. [Washington.]

Science—Science. New Series. [Washington.]

Serv. Bot. Bol. Téc. [Caracas]—Servicio Botánico. Boletín Técnico. [Caracas.]

Sierra Club Bull.—Sierra Club Bulletin.

Skr. Vid.-Akad. Oslo—Skrifter utgitt av det Norske Videnskaps-Akademi i Oslo. I. Matematisk-Naturvidenskapelig Klasse.

Smithson. Misc. Coll.—Smithsonian Miscellaneous Collections.

Soc. Bot. Mex. Bol.—Sociedad Botánica de México. Boletín.

Stain Tech.—Stain Technology. [The Commission on Standardization of Biological Stains.]

Sv. Bot. Tidsk.—Svensk Botanisk Tidskrift. [Svenska Botaniska Föreningen.]

Sv. Vet.-Akad. Handl. III—Kungliga Svenska Vetenskapsakademiens Handlingar. Tredje Serien.

Symb. Bot. Upsal.—Symbolae Botanicae Upsalienses. Arbeten från Botaniska Institutionerna i Uppsala.

Tech. Bull. Ariz. Exp. Sta.—Technical Bulletin. University of Arizona. College of Agriculture. Agricultural Experiment Station.

Tech. Bull. U. S. Dept. Agr.—Technical Bulletin. United States Department of Agriculture.

Trans. Acad. St. Louis—Transactions of the Academy of Science of St. Louis.

Trans. Am. Micr. Soc.—Transactions of the American Microscopical Society.

Trans. Am. Philos. Soc.—Transactions of the American Philosophical Society held at Philadelphia for promoting useful knowledge.

Trans. Ill. Acad.—Transactions of the Illinois State Academy of Science.

Trans. Kan. Acad.—Transactions of the Kansas Academy of Science.

Trans. Roy. Canad. Inst.—Transactions of the (Royal) Canadian Institute.

Trans. Wis. Acad.—Transactions of the Wisconsin Academy of Sciences, Arts and Letters.

Trav. Biol. Inst. Carnoy—Travaux biologiques de l'Institut J. B. Carnoy. [Louvain.]

Trillia—Trillia. Proceedings of the Botanical Society of Western Pennsylvania.

Trop. Woods—Tropical Woods. [Yale University, School of Forestry.]

U. S. Dept. Agr. Misc. Publ.—United States Department of Agriculture. Miscellaneous Publication.

Univ. Antioquia—Universidad de Antioquia. [Medellin.]

Univ. Ariz. Biol. Sci. Bull.—University of Arizona Bulletin. Biological Science Bulletin.

Univ. Buenos Aires Publ. Fac. Ci.—Universidad de Buenos Aires. Publicaciones de la Facultad de Ciencias Exactas, Físicas y Naturales. Serie B (Científico-técnica.)

Univ. Calif. Publ. Bot.—University of California Publications in Botany.

Univ. Colo. Stud. D.—University of Colorado Studies. Series D. Physical and Biological Sciences.

Univ. Ecuador Anal.—Universidad Central del Ecuador. Anales.

Univ. Iowa Stud. Nat. Hist.—University of Iowa Studies in Natural History.

Univ. Kan. Sci. Bull.—(The) University of Kansas Science Bulletin. [Title varies.]

Univ. Ore. Monogr. Bot.—University of Oregon Monographs. Studies in Botany.

Univ. S. Paulo Fac. Filos. Ci. Letr. Bol. Bot.—Universidade de São Paulo. Faculdade de Filosofia, Ciências e Letras. Boletim. Botânica.

Univ. Wash. Publ. Biol.—University of Washington Publications in Biology.

Univ. Wyo. Publ.—University of Wyoming Publications.

Va. Jour. Sci.—The Virginia Journal of Science.

Verh. Naturf. Ges. Basel—Verhandlungen der Naturforschenden Gesellschaft in Basel.

Wien. Bot. Zeits.—Wiener Botanische Zeitschrift. [Formerly Österreichische Botanische Zeitschrift.]

Wild Flower—Wild Flower. [The Wild Flower Preservation Society, Inc. Washington.]

Wrightia—Wrightia. [The Institute of Technology and Plant Industry. Southern Methodist University.]

INDEX TO AMERICAN BOTANICAL LITERATURE

COMPILED BY

LAZELLA SCHWARTEN

WITH THE COLLABORATION OF THE EDITOR OF THE TAXONOMIC INDEX

TAXONOMY, PHYLOGENY AND FLORISTICS

ALGAE

(See also under Spermatophytes: Camp, Rickett & Weatherby)

Daily, Fay Kenoyer. Species of *Tolypella* in Nebraska. Butler Univ. Bot. Stud. 8: 113–117. *1 pl.* O 1946 [Ap 1947].

Daily, William A. Notes on the algae—I, II. Butler Univ. Bot. Stud. 8: 118–120. *f. 1, 2.* O 1946 [Ap 1947].

Flint, Lewis H. Studies of freshwater red algae. Am. Jour. Bot. 34: 125–131. *f. 1–31.* Mr [Ap] 1947.

Smith, Gilbert M. On the reproduction of some Pacific coast species of *Ulva*. Am. Jour. Bot. 34: 80–87. *f. 1–38.* F [10 Mr] 1947.

Taft, Clarence E. Some algae, including new species, from New Mexico. Ohio Jour. Sci. 47: 85–87. *f. 1–4.* Mr [My] 1947. [New entities not validly published.]

Tseng, C. K. Seaweed resources of North America and their utilization. Econ. Bot. 1: 69–97. *f. 1–12 + tables 1–5.* Ja [Mr] 1947.

FUNGI AND LICHENS

(See also under Spermatophytes:
Camp, Rickett & Weatherby)

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LYCOPODIUM COPELANDIANUM, A SUMATRAN CLUBMOSS

RUTH CHEN-YING CHOU AND H. H. BARTLETT*

In describing a distinctive new species of *Lycopodium* from Sumatra in 1929, Copeland[1] inadvertently named it *L. petiolatum*, a binomial long pre-empted by a species of the Asiatic mainland. He stated that it was without similarity or near affinity to any other species known to him. It is clearly not the same as the older *Lycopodium petiolatum* and is therefore renamed. So clearly distinctive a species may well be named in honor of the eminent pteridologist who first described it, and we are therefore redescribing it as follows.

Lycopodium Copelandianum Chou & Bartlett, nom. nov. *L. petiolatum* Copeland, Univ. Calif. Publ. Bot. 14: 377. 1929; non Herter, Bot. Jahrb. Beibl. 98: 36. 1909.

Plants epiphytic, pendulous, rooting at the base only; branches up to 20 cm. long, 3–4 times dichotomously branched, 3–5 mm. diam. including the leaves, of nearly uniform diameter throughout the vegetative part; stems 1 mm. thick toward the base, 0.3–0.5 mm. toward the apex; phyllotaxy 6-farious, subverticillate with three leaves to each whorl alternating with those of the closely placed succeeding whorl; leaves small, fleshy-herbaceous, 2–3 mm. long, 1.2–1.5 mm. broad, ovate in outline if flattened but appearing falcate with both flanks of the blade retroflexed at the midrib, short-petiolate, decurrent; margin entire, slightly undulate; midrib obscure; stomata on the adaxial side of the leaf; epidermal cells elongate, wall sinuous; sporophylls largely in spikes, the lower ones only slightly different from the foliage leaves, subsessile, ovate lanceolate or triangular with rounded base, 2 mm. long, 1.0–1.2 mm. broad near the base, margin undulate, the upper ones distinct from the foliage leaves, aggregated into definite quadrangular, elongate, pointed cones 0.5–1.5 cm. long, 1.5–2.0 mm. diam., sessile, broadly ovate or suborbicular, pointed at apex, 1.2–1.5 mm. long, 1.2 mm. broad toward the base, with distinct, dorsally keeled midrib; sporangium sessile, reniform, 1 mm. broad, 0.5 mm. deep (from margin to sinus); outer cells of the sporangial wall elongate, 80–140 µ long by 20–30 µ wide, more or less regularly arranged with the long axis perpendicular to the margin of the sporangium, with undulate and uniformly thickened wall; spores trihedral, 28–32 µ diam., exospore minutely foveolate.

Dělěng Singkoet, near Běrastagi Karoland, Sumatra. (Plant geographers

A

B
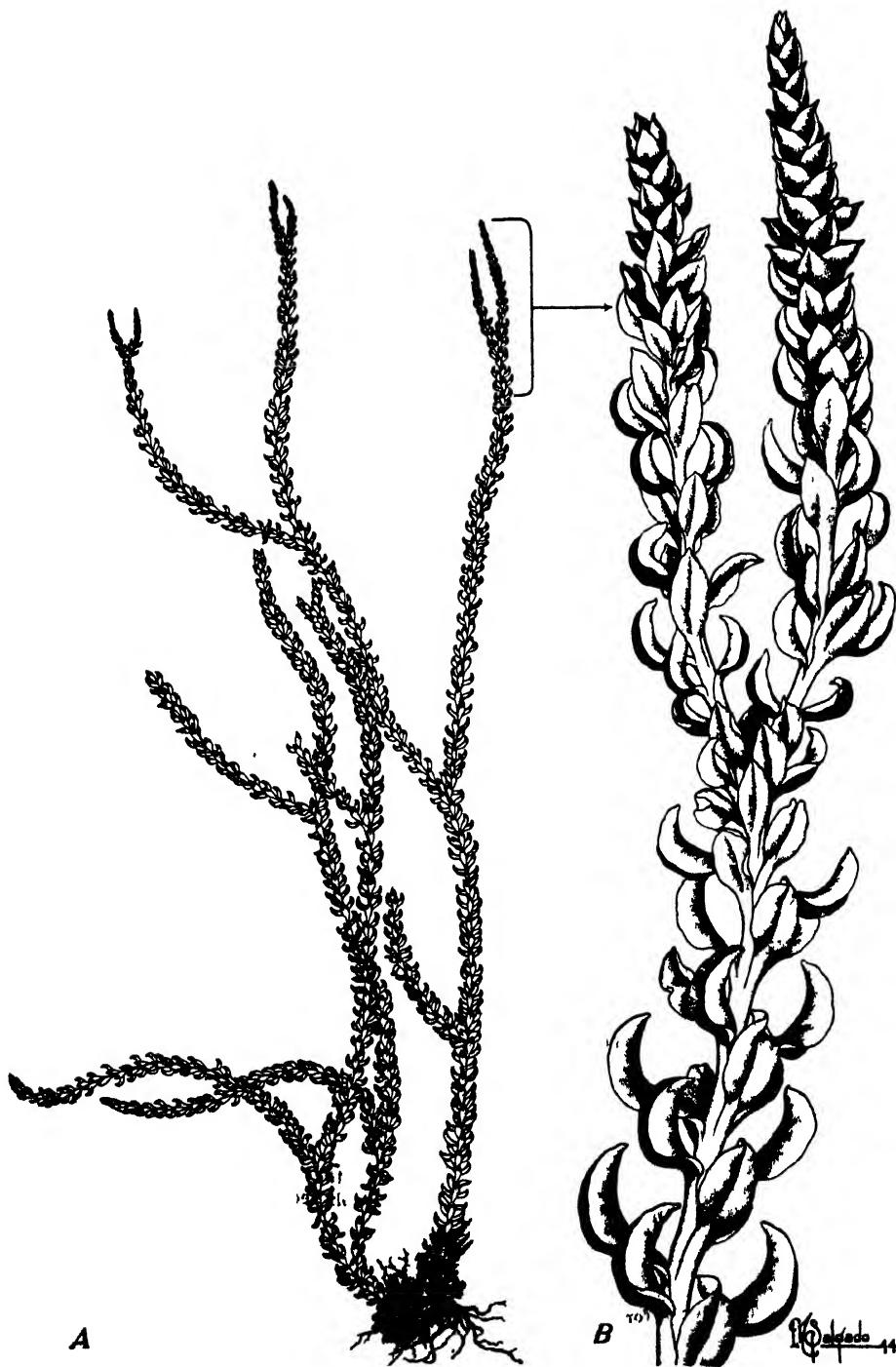
and ecologists will be interested in the reference to this locality in a former paper.[2])

The name *Lycopodium Hamiltonii* Spr. var. *petiolata* was proposed by C. B. Clarke[3] for a plant from Khasia. He cited no specimens, which presumably, were numerous, since he says: "Khasia, alt. 4000 ft.; frequent." It would be necessary to see the original material in order to select a specimen to stand as the type. This variety was maintained by Baker[4] for plants from the Himalayas, Kumaon, Moulmain, Lohfaushan, Neilgherries, Concan, and Ceylon. Whatever specimen were to be chosen as a lectotype would clearly have to be one conforming with Clarke's original description, from which we gather that it must have leaves ¼ to ⅓ in. (i.e., 6–8 mm.) long, of thin texture, and laxly scattered. Those of the Sumatran plant here described as *L. Copelandianum* are less than half that long and distinctly fleshy rather than thin. Himalayan *L. Hamiltonii* (not var. *petiolatum*) is described as having the leaves of the same length as those of the variety, although thick, and both species and variety have scattered, not subverticillate leaves.

Herter[5] in his study of Lycopodium in 1909, did not cite Clarke's original publication but raised the variety to specific rank on the basis of Baker's later publication, placing it with *L. Poisonii* in the incorrectly named Series "Poisonia" under subgenus *Urostachys*. Herter did not follow convexⁱonal procedure in making the new binomial, since he attributed it to Baker "pro var." in the systematic enumeration (l.c., p. 36) and did not cite the name-bearing synonym. It might therefore be argued that the binomial was not definitely established by him. In the index to his paper he gave the authority as "Bak. et Hert." The binomial has been taken up by other authors, however, and should be retained as *Lycopodium petiolatum* (C. B. Clarke) Herter, to avoid confusion.

In his formal enumeration of species Herter gives the distribution of *Lycopodium petiolatum* as the tropical Himalayan part of the Monsoon

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[2] **Bartlett, H. H.** The Batak Lands of North Sumatra, from the standpoint of recent American botanical collections. Univ. Philipp. Nat. Appl. Sci. Bull. 4: 211–323, *maps 1–2*. 1935.

[3] **Clarke, Charles Baron.** A review of the ferns of northern India. Trans. Linn. Soc. II. 1: 425–611. 1880.

[4] **Baker, J. G.** Handbook of the fern-allies. London, 1887. (See p. 9.)

[5] **Herter, W.** Beiträge zur Kenntnis der Gattung *Lycopodium*. Studien über die Untergattung *Urostachys*. Bot. Jahrb. Beibl. 98: 1–56. *f. 1–4. 4 tables*. 1909.

Explanation of figures A and B

Lycopodium Copelandianum. FIG. A. Habit of cotype in Herb. Univ. Mich., *Bartlett 6575*, showing subverticillate arrangement of lower leaves. × 0.65. FIG. B. Portion of branch showing gradual transition from vegetative leaves to sporophylls, and terminal strobili. (Sporangia are found on some leaves well below the specialized sporophylls of the strobilus.) × 4.
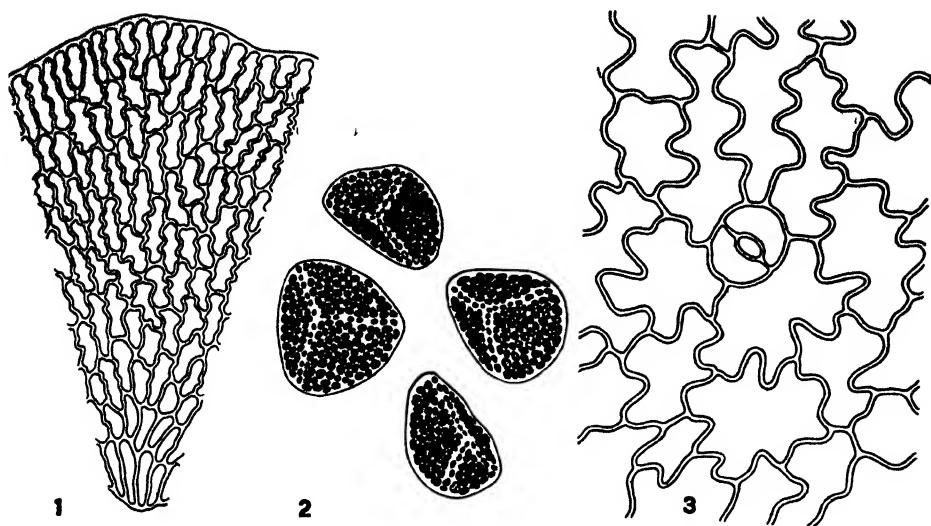
FIGS. 1–3. *Lycopodium Copelandianum.* FIG. 1: Portion of sporangial wall. × 95. FIG. 2. Spores. × 405. FIG. 3. Epidermal cells of upper (adaxial) side of leaf with stomata. × 225.

region only, but elsewhere (in the discussion of geographic distribution) he ascribes it also to Japan. The only species which he considers related to it he describes from Japan, as *L. Poisonii.*

Nessel[6] in his monograph of *Lycopodium* of 1939 (as stupidly muddled a work as often plagues the botanist) maintains "*Urostachys petiolatus*
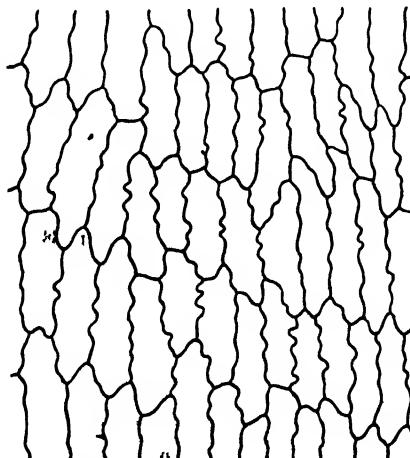
FIG. 4. Epidermal cells of lower side of leaf of *Lycopodium Copelandianum.* × 80.

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6 Nessel, H. Die Bärlappgewächse (Lycopodiaceae). Jena, 1939.

(Bak.) Hert., non C. B. Clarke'' as a species and also keeps it as a variety under *Lycopodium Hamiltonii*, separating the two by some 270 other species, varieties, and forms in the synopsis.   As a *Urostachys* he gives the wrong authority for the binomial, which, as far as there is any evidence, should be *U. petiolatus* (C. B. Clarke) Nessel. Whatever may be newly chosen as the actual (but originally uncited) type of Clarke's variety must also be the type of all the names created by Herter's and Nessel's inept juggling of Clarke's varietal name. Baker established no variety of his own, but merely maintained Clarke's. The same type can serve for only one of the two presumably distantly related plants which Nessel recognizes, for distant they must be if Nessel's classification represents natural grouping at all.

At any rate the binomial *Lycopodium petiolatum* (C. B. Clarke) Herter had been used, even though with the wrong authority, and had been accepted by other authors, prior to the publication of the homonym *L. petiolatum* Copeland. It becomes necessary, therefore, to give the latter a new name. Copeland's species was based upon a type specimen from Sumatra which does not correspond with the Indian plant known as *Lycopodium Hamiltonii* var. *petiolatum* or other names derived from that. Advantage is taken of the necessity for renaming Copeland's species to present an emended description, together with excellent drawings prepared by Mr. Eduardo Salgado from a cotype in the Herbarium of the University of Michigan (figs. A–B, 1–4).

DEPARTMENT OF BOTANY, UNIVERSITY OF MICHIGAN
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LYGODIUM FLEXUOSUM VAR. ACCIDENS, A NEW CLIMBING FERN FROM KWEICHOW PROVINCE, CHINA

RUTH CHEN-YING CHOU[*]

In the Gray Herbarium of Harvard University there is a single not too complete specimen of a fern which differs from plants considered to be *Lygodium flexuosum* (L.) Sw. chiefly in having the rachis nodose-articulate at the base of the petiolules. The characteristic is a definite and distinctive one, and it is to be presumed that the articulation provides for abscission of the leaflets, which are probably deciduous. It is questionable, however, whether the feature in question is one that should be used as a basis for specific separation of the plant. Nothing is known about the distribution of the type with articulately nodose rachis, of its constancy, or whether or not sufficient material would show other correlated distinctions to justify the description of a new species.

I have made a key to Chinese *Lygodia* on the basis of spore characteristics, and have found that the new variety here described from too young material may eventually be found to have additional characteristics. For the present, however, the matter is not certain and the type specimen is not so ample as one might wish.

Consequently, believing that the attention of botanists should be called to the existence of such a distinctive thing, but not wishing to establish a species on the basis of insufficient material, I am describing the peculiar plant as *Lygodium flexuosum* var. *accidens*. I am of the opinion, however, that some time, when more and complete specimens have been observed, it may be desirable to elevate the variety to specific rank. For this reason I give a detailed description of the type specimen, so that its identity may be clear to those who in the future may not have the advantage of interpreting it by examination of the type specimen.

LYGODIUM FLEXUOSUM (L.) Sw. var. **accidens** Chou, var. nov.   Axes (primarius et secondarii) straminei, nitidi, inferne subglabri vel pilis deciduis sparsim praediti, superne plus minusve pubescentes, marginibus acute costatis vel subalatis. Brachyrrhachis dichotomialis 5 vel 6 cm. longa, folia dua quasiopposita pinnate composita et ad dichotomiam gemmam dormientem terminatem pilosam ferens; pilis gemmae stramineis 0.5–1.0 mm. longis, 24–40 μ diam., subcylindricis gradatim ad apicem rectum vel curvatum angustatis ex cellulis 1 vel 2 basalibus et 2–4 ulterioribus constantibus;

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[*] Paper from the Department of Botany and Botanical Gardens of the University of Michigan.

foliis quasioppositis 20 cm. longis vel longioribus (petiolo incluso) pinnas 2–5 infra pinnam terminalem ferentibus; petiolo 3.5–4.0 cm. longo, plerumque bis longiore quam parte rhachios inter pinnam primam et secundam; rhachi nodose articulata ad petiolulorum basin; pinnis petiolulatis, basalibus palmate 3- vel 5-lobatis, lobo mediano quam lateralibus longiore, 4–6 cm. longo, 1.5–2.0 cm. lato (spicis marginalibus sporangialibus inclusis) basi valde cordato vel hastate auriculato; pinnis superioribus, 1- vel 2-lobis, basi sagittatis vel paululum cordatis, utrinque inconspicue pilosis, pilis brevibus, apiculatis, 1- vel pauci-cellulis cum aliis intermixtis clavatis glandularibus unicellulis; venulis tri- vel quadrifurcatis, utrinque aequaliter prominenti-
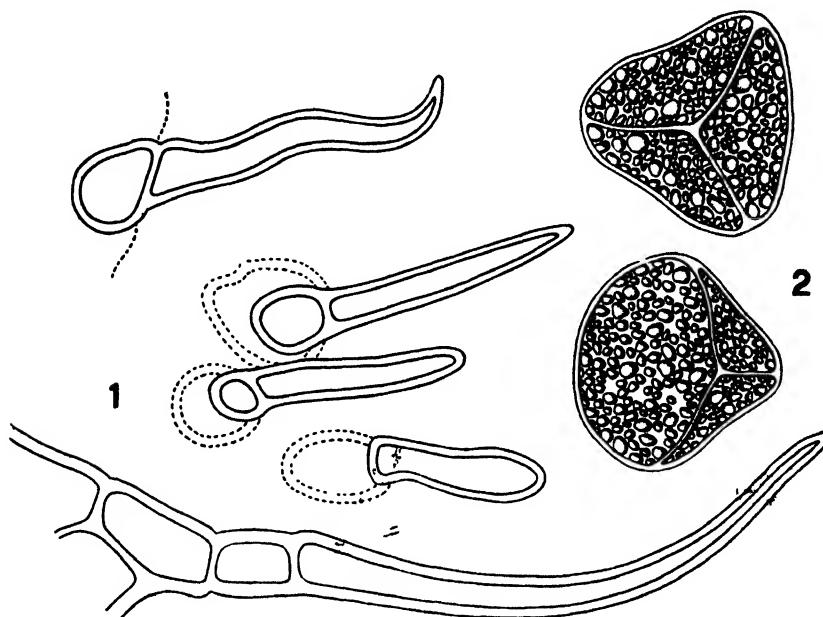
Figs. 1, 2. *Lygodium flexuosum* var. *accidens*. Fig. 1. Hairs from the epidermis. Fig. 2. Spores. Both figures × 605.

bus; plus minusve pubescentibus; margine pinnarum fertilium spicis sporangialibus densis monostichis ornata; margine pinnarum sterilium serrata, venulis ad apices dentium desinentibus; spicis nullis infra sinum; indusiis dense imbricatis sporangia ferruginea vel aurantiaca tegentibus; sporis pallide griseis, trihedricis, 60–85 µ diam. (saepissime 70–80 µ).

Typum legit Y. Tsiang, sub num. *7326,* 7 Oct. 1930 in silvis sparsis ad altitudinem 200 m. prope Chowshang in praefectura Changfeng, provincia Kweichow, Sina.

The type material was identified by R. C. Ching as *L. flexuosum,* to which it is likely of nearest affinity and under which I tentatively place it as a variety. However, its nodose-articulated rachis and petiolules (possibly with spore characteristics which are uncertain because of the immaturity of the specimen) justify giving it a name.
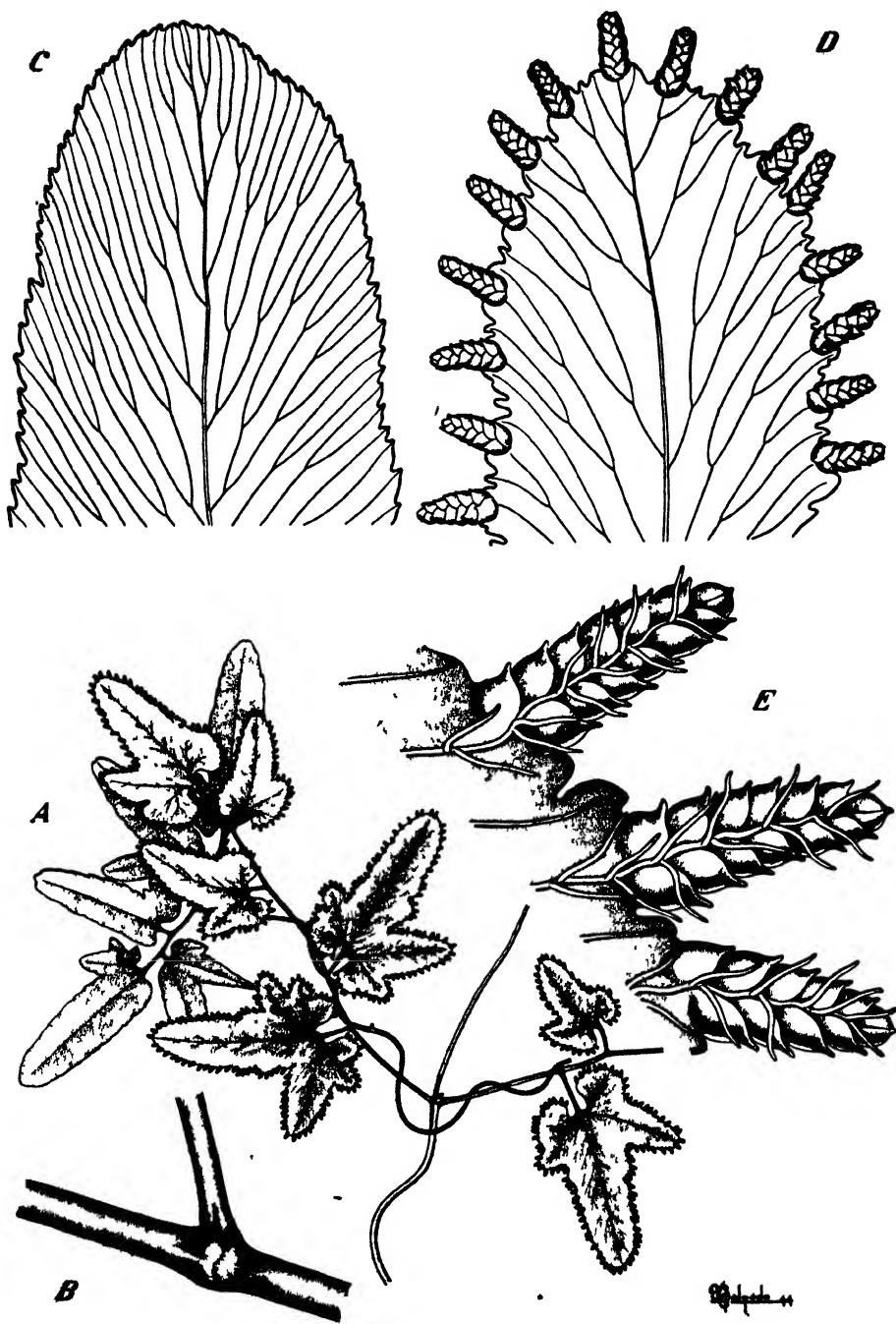
Taking into account the unsatisfactorily ascertained characteristics of the spores, this new variety would show affinity to *L. japonicum* (Thunb.) Sw. Thus one may surmise that if it is really a specific entity, its systematic position is intermediate between *L. japonicum* and *L. flexuosum*.

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Explanation of figures A—E

Lygodium flexuosum var. *accidens.* FIG. A. Habit of the type specimen, *Y. Tsiang 7326.* × 0.4.   FIG. B. Portion of rachis showing nodose articulation. × 5.   FIG. C. Apical portion of sterile pinnule showing veins and serrate margin. × 3.   FIG. D. Portion of fertile pinnule showing sporangial spikes (ventral view). × 3.   FIG. E. Sporangial spikes enlarged showing presence of hairs (ventral side). × 13.

SUPPLEMENTARY NOTES ON AMERICAN MENISPERMACEAE—IV

B. A. KRUKOFF AND H. N. MOLDENKE

Extensive collections of the Menispermaceae, largely from Amazonian Brazil, have recently become available to us. The collections examined extend our knowledge of certain species previously known to us from incomplete material; extensions of ranges are noted for a considerable number of species, and one species, *Abuta Soukupi* Moldenke, is described as new. No changes in nomenclature are necessitated.

The species are arranged in the same order and the place of deposit of specimens is shown by the same abbreviations as in our previous papers (1, 2, 3, 4, 5). The following new abbreviations are used:

DS: Dudley Herbarium, Stanford University, California.

IAN: Instituto Agronômico do Norte, Pará, Brazil.

MPU: Institut Botanique de l'Université de Montpellier, France.

CHONDODENDRON Ruiz & Pavon

1. CHONDODENDRON MICROPHYLLUM (Eichl.) Moldenke. Additional specimens examined: BRAZIL—BAHIA: *Curran 541* (G); basin of Rio Pardo, *Froes 12686, 12689a;* basin of Rio Santa Ana, *Froes 12701.*

2. CHONDODENDRON PLATIPHYLLUM (A. St. Hil.) Miers. Additional specimens examined: *Esc. Sup. Agr. & Vet. s.n. (Kr. Herb. 17734).*

3. CHONDODENDRON TOMENTOSUM Ruiz & Pav. Additional specimens examined: ECUADOR—ORIENTE: Rio Yatape Pacayaco, alt. ± 580 met., *Gill I* (DC). PERU—SAN MARTIN: San José de Ciza, *Massey s.n.*

5. CHONDODENDRON LIMACIIFOLIUM (Diels) Moldenke. Additional specimens examined: BRAZIL—AMAZONAS: basin of the upper Jurua, munic. Eirunepé, *Froes 21700, ? 21709, 21710, 21711, 21776, ? 21785, ? 21794.*

The first record of the species from the basin of Rio Jurua.

7. CHONDODENDRON TOXICOFERUM (Wedd.) Krukoff & Moldenke. Additional specimens examined: COLOMBIA—AMAZONAS: near Leticia, *Grassl 10076, Hermann 11309* (W.). PUTAMAYO: Rio San Miguel ó Sucumbios, *Schultes 3522.* PERU—IQUITOS: *Rosengarten I.* BRAZIL—AMAZONAS: basin of the upper Jurua, munic. Eirunepé, *Froes ? 21734, ? 21756, ? 21763, ? 21770, 21802a, 21836.*

The first record of the species from Colombia. The vernacular name "sa-pĕ-pa" for the species is recorded by Schultes, who also notes on his label that "the wood of stem crushed to make curare poison."

SCIADOTENIA Miers

2. SCIADOTENIA PARAËNSIS (Eichl.) Diels. Additional specimens examined: BRAZIL—PARÁ: near Belém, *Murça Pires & Black 531.*

9. SCIADOTENIA CAYENNENSIS Benth. Additional specimens examined: BRAZIL—PARÁ: *collector undesig. s.n.* (MPU).

10. SCIADOTENIA BRACHYPODA Diels. Additional specimens examined: BRAZIL—AMAZONAS: basin of the upper Jurua, munic. Eirunepé, Mucambinho Revalisa, *Froes 21834.*

12. SCIADOTENIA SPRUCEI Diels. Additional specimens examined: BRAZIL —AMAZONAS: *Ducke 854.*

13. SCIADOTENIA TOXIFERA Krukoff & Smith. Additional specimens examined: PERU—IQUITOS: Huyabamba, *Murça Pires & Black 870;* near Iquitos, *Rosengarten 2.*

The species has been known to date from two sterile collections from Ecuador. The specimen is the first one known with staminate inflorescences. A description of the flowers follows: staminate flowers; sepals about 14, very thick-textured, leathery, firm, ovate, convex and densely appressed-strigose on the outer surface, concave and smooth on the inner surface, acute at apex, closely imbricate in 3 or more series, the outermost minute, about 0.6 mm. long and 0.5 mm. wide, the innermost ca. 2 mm. long and 1.7 mm. wide, the remainder intermediate in size; petals 6, broadly spatulate, very fleshy, erect, firm, ca. 1 mm. long and 0.8 mm. wide, rounded at apex and at the apex of the 0.2 mm.-long claw, thickened into 2 parallel, very fleshy, median, erect ridges or humps extending from the apex of the claw to beyond half-way up to the apex of the expanded portion of the petal, so closely adjacent as to practically touch, all the petals appressed-pilose on the back and ciliate on the margins; stamens 6, connate at the base, the connate portion 0.6–0.7 mm. long, glabrous, terete, the free portions ca. 0.8 mm. long in all, glabrous; anthers lateral, light colored, horizontally dehiscent, ca. 0.6 mm. wide.

ANOMOSPERMUM Miers

1. ANOMOSPERMUM SCHOMBURGKII Miers. Additional specimens examined: BRAZIL—PARÁ: near Santarem, *Ducke 16378* (PG). VENEZUELA— BOLIVAR: *Steyermark 59220* (F, N), *60623* (F, N), *60565* (F, N).

The first record of the species from Venezuela.

3. ANOMOSPERMUM RETICULATUM (Mart.) Eichl. Additional specimens examined: COLOMBIA—AMAZONAS: Loretoyaco, *Black & Schultes 46–161.* BRAZIL—AMAZONAS: basin of the upper Jurua, munic. Eirunepé, Adelia, *Froes 21822a.* PARÁ: basin of Rio Jamundá, *Ducke 11733* (PG).

The first record of the species from Colombia and from the basins of the rivers Jamundá and Jurua in Brazil.

6. ANOMOSPERMUM CHLORANTHUM Diels. Additional specimens examined: ECUADOR—AZUAY: "in region of Sanaguin, in rich rain forest jungle," *? Steyermark 52749* (F, N).

TELITOXICUM Moldenke

1. TELITOXICUM KRUKOVII Moldenke. Additional specimens examined: BRAZIL—AMAZONAS: basin of the upper Jurua, munic. Eirunupé, *Froes 21789.*

The first record of the species from the basin of Rio Jurua.

5. TELITOXICUM DUCKEI (Diels) Moldenke. Additional specimens examined: BRAZIL—BAHIA: basin of Rio Santa Ana, munic. Ilheos, *Froes 12718.*

This is the first record of the genus from the State of Bahia.

ABUTA Barrère

1. ABUTA MACROCARPA Moldenke. Additional specimens examined:
BRAZIL—AMAZONAS: basin of the upper Jurua, *Froes 21768*.
The first record of the species from the basin of Rio Jurua.

2. ABUTA OBOVATA Diels. Additional specimens examined: VENEZUELA—
BOLIVAR: between Santa Teresita de Kavanayen and Rio Pacairao (tributory
of Rio Mouak), *Steyermark 60395* (F, N).
The first record of the species from Venezuela.

3. ABUTA BULLATA Moldenke. Additional specimens examined: BRAZIL—
AMAZONAS: basin of the upper Jurua, munic. Eirunepé, *Froes 21699, 21755,
21796*.
The first record of the species from the basin of Rio Jurua.

9. ABUTA COLOMBIANA Moldenke. Additional specimens examined: CO-
LOMBIA—VALLE DEL CAUCA: *Cuatrecasas 14028*.
 -   The first record of the species from Valle del Cauca.

11. ABUTA GRANDIFOLIA (Mart.) Sandw. Additional specimens ex-
amined: COLOMBIA—AMAZONAS: basin of Rio Putumayo, *Schultes 3740*.
BRAZIL—AMAZONAS: basin of Rio Solimoes, *Ducke 360* (A).

13. ABUTA SELLOANA Eichl. Additional specimens examined: BRAZIL—
MINAS GERAES: *Esc. Sup. Agr. & Vet. 1632, 3532*.

14. ABUTA RUFESCENS Aubl. Additional specimens examined: VENE-
ZUELA—MERIDA: along Rio Onia, near Bolero, *Steyermark 56700* (F, N).
BRAZIL—AMAZONAS: basin of the upper Jurua, municipality Eirunepé, *Froes
21708, 21754, 21758, 21764*. PARÁ: near Belem, *Murça Pires & Black 661*.
MINAS GERAES: *Esc. Sup. Agr. & Vet. 1630*.
The first record of the species from Venezuela and from the basin of Rio
Jurua in Brazil.

16. ABUTA GRISEBACHII Triana & Planch. Additional specimens ex-
amined: BRAZIL—AMAZONAS: basin of Rio Negro ("Jauaraté"), *Froes
21277* (IAN); near Parintins, *Murça Pires & Black 1149*.

17. ABUTA CANDOLLEI Triana & Planch. Additional specimens examined:
BRAZIL—PARÁ: Belterra, *Baldwin Jr. 2752* (N, W).

18. ABUTA SPLENDIDA Krukoff & Moldenke. Additional specimens ex-
amined: VENEZUELA—MERIDA: along Rio Onia, near Bolero, *? Steyermark
56701* (F, N). COLOMBIA—PUTUMAYO: Rio San Miguel o Sucumbios, Conejo
y los alredadores, *Schultes 3525*.
The first record of the species from Colombia and Venezuela. The vernacu-
lar name "sa-pĕ-pa" for the species is recorded by Schultes, who also notes
on his label that the plant is used in preparation of curare.

22. Abuta Soukupi Moldenke, sp. nov. Planta lignosa; ramis gracilibus
longitudinaliter striatis carinatisque glabris; foliis alternis, supra cicatricem
ferruginoso-tomentosis; petiolis gracilibus 7–12 mm. longis non incrassatis
obscure pilosulis glabrescentibus; laminis firme membranceis anguste ellipti-
cis 8–15 cm. longis, 2–4 cm. latis, acuminatis integris, ad basin cuneatis,
utrinque glaberrimis pernitidisque; venis secundariis 2; venis venulisque
utrinque argute prominulis; inflorescentiis ♂ anguste racemosis foliosis;
floribus subumbellatis.

Woody plant; branches slender, longitudinally striate and sharply ridged
for 1–2 cm. below each leaf scar (at least at and near the tips of the

branches), glabrous except just above each leaf-scar where there is a short longitudinal band of dense ferruginous-tomentose hair; leaves alternate; petioles slender, 7–12 mm. long, very obscurely scattered-pilosulous, becoming glabrous, not enlarged at base nor apex, flat on the upper (axial) surface; blades firmly membranous, narrow-elliptic, 8–15 cm. long, 2–4 cm. wide, acuminate at apex, entire, cuneate at base, glabrous and very shiny on both surfaces; midrib slender, sharply prominulous on both surfaces; secondaries one pair, issuing at the very base of the leaf, exactly similar to the midrib in texture and prominence on both surfaces, extending to 2 or 3 cm. from the apex and there ending in many loops; tertiaries very numerous, extending at almost right angles from the midrib and secondaries and connecting them, they and the veinlet-reticulation uniformly slender and sharply prominulous on both surfaces, only slightly less elevated than the secondaries and midrib; staminate inflorescence axillary or supra-axillary, toward the tips of the branches, 1–3 per axil, 3.5–10 cm. long, regularly 1.5–2 cm. wide throughout, racemose, the longer ones often bearing one or more leaves which are similar to the stem-leaves in all respects except only to 8.5 cm. long and 2 em. wide; rachis slender, continuous and uniform in thickness from base to apex of raceme, straight, firm, more or less short-pilose with ferruginous or yellowish hairs, especially toward the base, glabrescent in age, with dense tufts of short hair at the base of each side-branch; side branches of the raceme very slender, firm, straight, 5–8 mm. long, widely divergent, slightly pilosulous or glabrescent, bearing at their apex a dense many-flowered umbelloid cluster of flowers; pedicels slender, 1–3 mm. long; flowers wide open in anthesis, with the anthers very conspicuous: the 3 outer sepals membranous, light, wide-spreading or reflexed, concave (if at all) on the outer face, about 1 mm. long, elliptic, acute at apex, glabrous; the 3 inner sepals membranous, light, translucent at the reflexed subhyaline margins and apex, obovate-elliptic, about 1.7 mm. long and 1.3 mm. wide, glabrous; the 6 stamens in two groups of 3 each, each group widespreading from the center, about 1 mm. long; filaments dark, much more slender than the anthers; anthers yellow.

The type of this species was collected by Jaroslaw Soukup, S.S. (*1466*), at Lima, Peru, in 1941, and is *1124877* in the herbarium of the Chicago Natural History Museum. The species is apparently closely related to *A. grandifolia* (Mart.) Sandw. which differs in the following characters: leaves much larger and heavier; midrib and secondaries stouter, not as sharp on either surface; tertiaries and veinlet reticulation not prominulent on either surface, at least, not sharply so; petioles with a usually well-developed pulvinus at the apex and base; branches round in cross-section and completely glabrous throughout, without bands of tomentose hair at the leaf-scars; inflorescence completely glabrous throughout, without tufts of hair, very irregular in size and shape, usually in large clusters in the leaf-axils, very loose and open; flowers borne singly or in pairs on pedicels of very irregular length, not at all umbellate-clustered; the 3 outer sepals thick-textured, opaque, lanceolate, concave on the inner face, convex on the outer face, about 0.8 mm. long and 0.4 mm. wide, obtuse at apex, microscopically puberulent; 3 inner sepals thick-textured, opaque, elliptic-suborbicular, concave on the inner face, convex on the outer face, about 1 mm. long and 0.8 mm. wide, rounded or obtuse at apex, microscopically puberulent; the

6 stamens central, erect, parallel, about 0.6 mm. long, dark, their filaments thick, not conspicuously thinner than the anthers.

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A COLLECTION OF MARINE ALGAE FROM BRAZIL

Louis G. Williams and H. L. Blomquist

While stationed with various Naval Air Facilities in Brazil during parts of 1944 and 1945, the senior author had an opportunity of collecting a limited number of marine algae from this South American republic. Of course, other activities in the line of duty as well as lack of certain facilities prevented any extensive study. But, on the other hand, having facilities of the Navy for travel afforded certain advantages, at least for making observations. Practically the whole coast line of Brazil, extending over a distance of some 3,700 miles and embracing 36 degrees of latitude, was viewed either from high-flying planes, or low, more slowly-moving blimps. Also, the shore was observed at close range from the ground at various places, such as São Luiz, Fortaleza, Natal, Recife, Maceio, Bahia, Rio de Janeiro, and Santos.

It was obvious from these observations that much of the shore line of northern Brazil as far south as Natal must be mostly barren of littoral algae because of the lack of suitable habitats. For one thing, the gigantic Amazon pours into the ocean a tremendous volume of muddy fresh water, especially during flood season, which spreads out on both sides of its estuary for hundreds of miles along the coast, creating muddy shores and extensive fresh or brackish marshy areas. Secondly, other portions not affected by fresh water apparently lack exposed reefs or rocky shore-lines but consist mostly of long stretches of barren sandy or muddy beaches, which are unsuitable habitats for attachment of algae.

These conclusions on the scarcity of algal habitats on the coast of northern Brazil are supported by the paucity of records in the literature on littoral algae from this area. In a synopsis of the records of marine algae from Brazil (Taylor 1930), only two species are listed as having come from north of Fortaleza, located about 750 miles south of the Amazon estuary. Near Fortaleza, however, is a rocky cape (Mucuripe) which has apparently furnished some algae (36 spp.), but from there to Natal the coast is again a barren, sandy beach.

The approximately 3,700 miles of Brazilian shore-line may be roughly divided into three distinct regions. The northernmost 1,350 miles consist mostly of sandy or muddy beaches and the wide Amazon estuary. This area is apparently almost devoid of littoral algae. The middle portion of some 500 miles has an offshore reef consisting of consolidated sandstone which furnishes ideal habitats for many algae. The extensive southern coast of about 1,850 miles has several stretches of rocky shores, which also offer favor-

able substrata for attached algae. It is mainly from this southern part of the coast that most of the material has come upon which our previous knowledge of the marine algae of Brazil is based.

The sandstone reef which skirts the middle portion of the coast of Brazil, extends, with some interruptions, from approximately the northern boundary of the state of Pernambuco through the vicinity of Maceio to some distance north of Aracaju. The reef varies in its distance from shore, extent to which it has been eroded, and exposure above the sea. Large portions of it are exposed at low water and in many places it is even exposed at high tide (fig. 1). In several places outside of certain estuaries where it acts as a breakwater this reef has made harbors possible. This is the situation especially at Recife, which has received its name from this reef.

FIG. 1. Portion of sandstone reef south of Recife, Pernambuco, Brazil.

The reef itself is not only an excellent habitat for algae but also the channel or lagoon between it and the beach. From the standpoint of algal habitats it reaches its best development just south of Recife. The majority of algae listed below were collected at this locality. Collecting was done during the first four months of 1945, but mainly during February and March. No doubt other species might have been obtained from the nearby estuaries and mangrove swamps, but because of the danger of infection from the heavily polluted water no collecting was attempted in such places.

While no special study was made of distribution of species in relation to habitats, some impressions regarding this were gathered from general observations while collecting. The habitats may be generally classified into (1) those pertaining to the reef itself, and (2) those associated with the reef.

Because of erosion by wave action the reef varies greatly in relief, exposure to air, and wave action. The surface is in general uneven and, in many places broken or deeply fissured (fig. 1). The ocean side is exposed to severe wave action while the channel side is more or less protected. All of these variations offer a variety of habitats for algal attachment and growth.

Growing on the upper ledges of the steep outside parts of the reef and hence subjected to constant pounding by waves as they break over the reef are three species of *Gracilaria* (*G. ferox*, *G. cornea*, *G. ornata*), *Bryothamnion Seaforthii*, *Digenea simplex*, *Vidalia obtusiloba*, and *Sargassum polyceratium*. Ledges in deeper water—three or more feet below low tide level—are frequented by three species of relatively large brown algae, *Spathoglossum Schröderii*, *Dictyopteris Justii*, and *Zonaria zonalis*. Others associated with these are *Halimeda discoidea* and *Liagora ceranoides*.

Where the reef does not rise abruptly but slopes gradually outward and wave action is less severe, such species as *Gracilaria cervicornis*, *Botryocladia Uvaria*, and *Laurencia scoparia* grow abundantly. Below this are reddish-brown mats of *Bostrychia Binderi* and *Hypnea spinella* interwoven with filaments of *Lophosiphonia obscura*.

Over the top of the reef where it is exposed between waves, especially at low tide, is a dense, dull-green carpet of various species of which the following are typical: *Laurencia papillosa*, *L. obtusa*, *Acanthophora spicifera*, and *Amansia multifida*, usually interwoven with a growth of the wiry *Gelidium rigidum*.

Attached to the walls of the transverse and diagonal fissures in the reef and hence protected from strong light and desiccation at low tide are *Anadyomene stellata*, *Valonia ventricosa*, *Dictyosphaeria cavernosa*, and *Cladophora membranacea*.

On the inside walls exposed between waves at low tide is a virtual paradise for the genus *Caulerpa*. Here the following five species grow luxuriantly: *C. crassifolia* f. *mexicana*, *C. Lycopodium*, *C. prolifera*, *C. sertularioides*, and *C. racemosa* var. *uvifera*.

The protected areas in the lagoon between the reef and the sandy beach support a variety of species. Here the bottom is composed of rock or silt and the plants are protected from strong waves. The nature of the bottom largely determines the quality of the flora. Here are found beds of *Ulva*; *U. fasciata* being the most abundant and *U. Lactuca* var. *rigida* less so. The former extends in places up on the walls of the reef. Two species of *Padina* (*P. gymnospora* and *P. Vickersiae*) grow in beds of silt. There are also small, scattered patches of *Dictyota cervicornis* and *Dilophus alternans*. Growing in shallow rock pools just below low tide level is an abundance of a unique rugged form of *Hypnea musciformis* and frequently beds of *Sargassum platycarpum*. Similar habitats are also frequented by *Gracilaria cornea* and

G. cylindrica, which are fed upon by herds of sea hares which, when disturbed, interfere with collecting by discharging a deep purplish exudate. Small green patches of *Caulerpa pusilla* were found in silt where plants are protected by the reef wall. *Halymenia Floresia* and *H. floridana* together with *Nemalion Schrammii* grew attached to a rock bottom in some places between two parallel-arranged reefs. Such places are also inhabited by *Codium isthmocladum* and *C. dichotomum.*

In addition to the collection from the reef off Pernambuco south of Recife, a small collection was obtained also from the island of Fernando Noronha which lies some 250 miles northeast of Natal.

As has been noted by other investigators (Howe 1928; Taylor 1930), the littoral marine algae of Brazil show strong affinities with those of the West Indies. This is especially evident from the entities reported here. Some forms even range as far north as North Carolina. It is also interesting to note that there is evidently some floristic relation between the algal flora of Brazil and the south Pacific, as has been pointed out by Børgesen for the West Indies (1915–20, 1924).

A total of 116 entities have been identified from this collection. Of these, 33 are new to northern Brazil, and 22 are reported for the first time from Brazil.

MYXOPHYCEAE
Coccogonales—Chamaesiphonaceae

DERMOCARPA PRASINA (Reinsch.) Born. & Thuret.[1] Epiphytic on *Rhizoclonium riparium* and other algae of the reef. Reported here for the first time from Brazil. W. I., Bermuda and Florida.

Hormogonales—Oscillatoriaceae

OSCILLATORIA CORALLINAE Gomont. Epiphytic on *Dictyosphaeria cavernosa* from reef crevices. Reported here for the first time from Brazil. W. I. to Florida and Bermuda.

CHLOROPHYCEAE
Ulvales—Ulvaceae

ULVA FASCIATA Delile. The plants of this species collected on the reef and at Fernando are smaller than *U. Lactuca* var. *rigida* and are not stiff and brittle when dried. There is no tendency for the thallus to be pinnately lobed, but a study of the cross section reveals cells of the center of the blade to be about three times as long as broad and becoming shorter toward the margin. This species grows profusely in beds of silt just below low tide level and on the reef in places where it is not exposed at low tide. Tropical waters the world over.

ULVA LACTUCA L. Same habitats as the preceding species but relatively uncommon. The cells viewed in cross section of the thallus are about as long

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[1] Determined by Dr. Francis Drouet.

as wide with no gradation in size from the middle toward the margin. Plants not brittle when dried. World wide in distribution.

ULVA LACTUCA var. RIGIDA (C. Ag.) LeJolis. The most common member of the genus in the areas collected about the reef and at Fernando where it forms large beds of well developed plants. The spécimens are very thick and coarse, becoming brittle when dried. It has been previously reported from nearly every station where algae have been collected in Brazil. Common in warm waters of the western Atlantic Ocean.

Siphonocladiales—Cladophoraceae

CHAETOMORPHA GRACILIS Kütz. Common at Recife and Fernando, entangled with other algae, especially *Gelidium rigidum* and *Digenea simplex* in the intertidal zone. Previously reported from Rio de Janeiro, but reported here for the first time from northern Brazil. W. I., and Florida.

RHIZOCLONIUM RIPARIUM (Roth.) Harv.[2] Common in mats of *Bostrychia Binderi* from the reef and Fernando Reported here for the first time from Brazil. W. I. Probably widely distributed.

CLADOPHORA GRACILIS (Griffiths) Kütz. f. SUBFLEXUOSA Coll.[2] Growing on wall of a cliff exposed intermittently between waves on Fernando. So far as is known, this is the first report of this species south of Florida. General distribution unknown.

CLADOPHORA MEMBRANACEA (C. Ag.) Harv.[2] *Cladophoropsis membranacea* (C. Ag.) Børg. In tufts on large rocks at Fernando and abundantly in depressions and on the inside wall of the reef. Exposed during low tide. This is the first report of this species from Brazil. Common in the W. I. to Florida.

Valoniaceae

ANADYOMENE STELLATA (Wulf.) C. Ag. An uncommon species on the reef. One specimen was found growing in a transverse fissure of the reef just below low tide level. W. I., Bahamas, Bermuda, to Florida.

DICTYOSPHAERIA CAVERNOSA (Forssk.) Børg. Adhering to the inner wall of the reef and in crevices, and at base of boulders at Fernando. A member of the algae of the lower intertidal zone. This species has been reported once previously from Brazil. W. I., Bahamas, Bermuda, to Florida.

CHAMAEDORIS PENICULUM (Ell. & Sol.) Kunze. Growing on silt in lagoon between reef and beach. W. I., Bermuda to Florida.

VALONIA VENTRICOSA J. Ag. One specimen was found concealed in a crack in the reef. This species has been found previously in the Abrolhos Islands in southern Brazil. W. I., Bahamas, Bermuda, Florida.

Siphonales—Bryopsidaceae

BRYOPSIS PENNATA Lamour. Very common in depressions in the reef retaining water during low water. W. I., Bahamas, Bermuda, Florida to North Carolina.

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[2] Determined by Dr. Harry K. Phinney.

Caulerpaceae

CAULERPA CRASSIFOLIA (C. Ag.) f. MEXICANA (Sond.) J. Ag. Commonly washed ashore on Fernando and very abundant on the inner wall of the reef giving it a light-green color. It develops here into large plants, the erect branches attaining a length of 9 cm. from extensive stolons. Collectors in the West Indies report it from considerable depths. In Pernambuco it thrives at low tide levels. The form of this species is reported here for the first time from Brazil. W. I., Bermuda, Florida.

CAULERPA LYCOPODIUM J. Ag. (Figure 2) This alga superficially appears like a club moss growing on the inner wall of the reef in the intertidal zone.
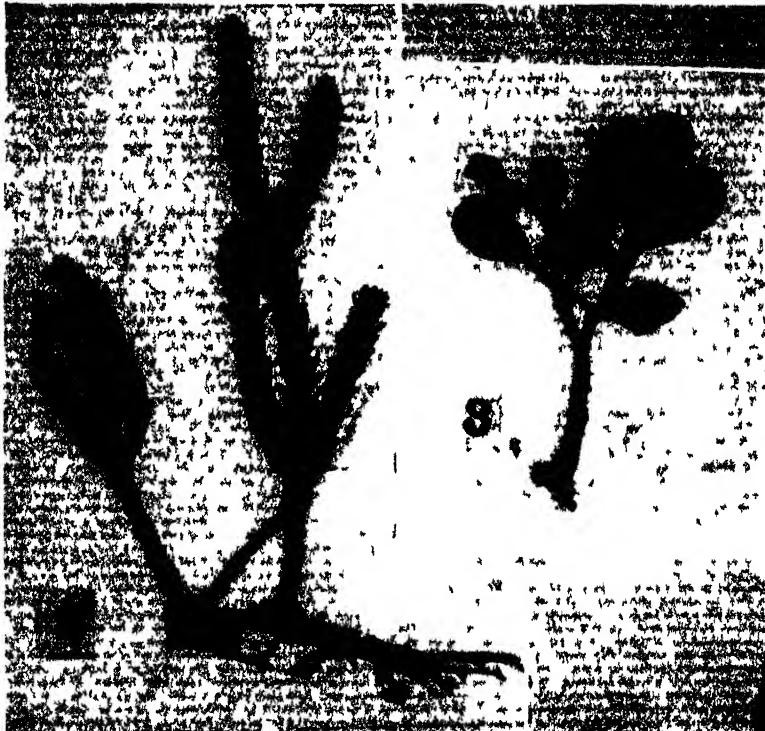
FIG. 2. *Caulerpa Lycopodium* J. Ag. × 1   FIG. 3 *Botryocladia Skottsbergii* Børg. × 1.25.

It was at first mistaken for *C. lanuginosa* J. Ag., with which it has been previously confused. It differs, however, from the latter in having entire ramuli and in the absence of branched hairs on the stolons and erect branches. The ramuli are relatively large with mucronate tips. The erect branches frequently become 15 cm. long. No records have been found of recent collections of this species, but DeToni reports it from Bahia and from Martinique.

CAULERPA PROLIFERA (Forssk.) Lamour. The blades of the thallus of this alga protrude from between other algae covering the various surfaces of the

reef, particularly the inner wall, where some of the plants are exposed during low tide. It was also found growing in silt between large rocks at Fernando. From the Caribbean area Taylor (1942) reports this alga in water of moderate depths, but in Fernando it thrives in the intertidal zone. W. I., Bahamas, Bermuda, Florida.

CAULERPA PUSILLA (Kütz.) J. Ag. This alga was found growing in small green patches in silt in a protected bay at Fernando and in the same kind of habitat between the reef and the beach in Pernambuco. The plants were bright green when collected, but turned dark-red on drying. This species is coarser than *C. verticillata* J. Ag. and has shorter erect branches (about 1 cm. tall) with ramuli more numerous in the whorls, and with numerous short forked hairs. Barbados.

CAULERPA RACEMOSA (Forssk.) J. Ag. var. LAETEVIRENS (Mont.) Weber-van Bosse. This variety grows on both sides of the reef below tide level, and was also found growing from a rocky bottom at Fernando. This is the largest Caulerpa encountered in Brazil. It grows in dense bunches with erect branches sometimes becoming 1 cm. in diameter and 13 cm. long. W. I., Bermuda and Florida.

CAULERPA RACEMOSA (Forssk.) J. Ag. var. CLAVIFERA (Turn.) Weber-van Bosse. This variety grows in more exposed places than any of the other Brazilian caulerpas encountered. In places where it is found on top of the reef it is subjected to considerable drying. It was also found free floating at Fernando. It is a smaller plant than the preceding variety. W. I., Bermuda and Florida.

CAULERPA RACEMOSA (Forssk.) J. Ag. var. UVIFERA (Turn.) J. Ag. This is the smallest of the racemose Brazilian caulerpas. It is found attached to the inner wall of the reef and in less exposed places on top of the reef where it loses its green color (apparently from excessive rains, which bring more freshwater than it can tolerate while exposed to the air) and becomes yellow. Here reported for the first time from northern Brazil. W. I., Bermuda and Florida.

CAULERPA SERTULARIOIDES (Gmelen) Howe. This plumose alga becomes a large plant on the reef and at Fernando, where the erect branches frequently measure 12 cm. in length, and 1.5 cm. wide, growing from an extensive stoloniferous system at low tide levels and protruding from other algae on the inner wall of the reef, or from silt at base of rocks on Fernando. Some of the plants approach the *longiseta* form [*C. sertularioides* f. *longiseta* (Bory) Svedel.], but there are so many gradations to the larger sizes it was decided to place them all in the species. Reported for the first time from northern Brazil. Barbados, Bermuda and Florida.

CAULERPA WEBBIANA Mont. f. TOMENTELLA (Harv.) Weber-van Bosse. Frequently found free floating and entangled with other algae. One cluster was found growing attached to an *Amphiroa fragillissima* in a transverse crevice of the reef. Previous collectors (Børgesen 1913–1914, Collins 1909) from other localities report this species from deep water (to 50 m.). In Pernambuco it was found at low tide levels. W. I., Virgin Islands and Florida.

Codiaceae

CODIUM DICHOTOMUM (Huds.) S. F. Gray. One bed of this species was found on a rock bottom about 5 ft. below low tide level between two -parallel-running reefs. However, since large quantities were washed ashore both in Pernambuco and on Fernando Noronha it must be considered common. The utricles average over 180 μ and the tips are moderately thickened. When dried the plants have a wooly appearance. This species has been found wherever algae have been collected in quantities in Brazil and is cosmopolitan in warmer waters.

CODIUM ISTHMOCLADUM Vick. Found among drifting algae near the shore at Fernando. The plants are long and slender, constricted at the bases of the branches, and light green in color. This report is new to Brazil, but this species is known from Venezuela, W. I., Bermuda and Florida.

HALIMEDA DISCOIDEA Decaisne.   Scattered plants were found growing on the slopes of the ocean side of the reef in about 3 ft. of water below low tide levels. Since large numbers of plants were found washed ashore both in Pernambuco and on Fernando, it must be considered common in deeper water than the reef algae. This is the first report of this species from Brazil. W. I., Bahamas to Florida.

HALIMEDA OPUNTIA (L.) Lamour. A few specimens were found lodged in crevices in the reef and many fragments were found drifting ashore in both Pernambuco and Fernando. It is probably common in offshore waters. Caribbean area, Bermuda and Florida.

HALIMEDA SIMULANS Howe. In Pernambuco this species was commonly found growing in silt in crevices of the reef sloping toward the ocean about 2 feet below low tide levels. This is the only species of *Halimeda* collected ,in this area that is common in the shallow water associated with the reef. On Fernando it was growing in muddy silt between the shore and nearby offshore rocks. It was also common among the algae washed ashore. This is the first report of this species from Brazil, but it has been reported previously from Columbia, Caribbean, Gulf of Mexico, and Tropical North Atlantic.

HALIMEDA TUNA (Ell. & Sol.) Lamour. Only one specimen of this species was collected in Pernambuco, growing with *H. discoidea*. Barbados to Bermuda.

PENICILLUS CAPITATUS Lamarck. In Pernambuco, growing on silt in the lagoon between the reef and the beach, and frequently so masked by silt that it was easily overlooked. Because the stipes are shorter than the capitulum the plants of this collection might be considered a new form. The average length of stipe is 13 mm., and the average branching filament of the capitulum is 16 mm. long. The diameter of the filaments of the capitulum is about 180 μ, which would place it in the range of the species to which it is assigned here. This is the first report of this genus and species from Brazil. It has been reported previously from Venezuela to Bermuda.

UDOTEA FLABELLUM (Ell. & Sol.) Howe. In Pernambuco growing on silt in the reef-formed lagoon below low tide level. Washed ashore on Fernando. This species has been reported once previously from Brazil in a northern locality. It is known from as far north as North Carolina.

PHAEOPHYCEAE

Dictyotales—Dictyotaceae

DILOPHUS ALTERNANS J. Ag. A few plants drifted ashore on Pernambuco. Barbados to Bahamas and Florida.

DICTYOTA CERVICORNIS Kütz. In Pernambuco this species grows in abundance in the reef-formed lagoon on a shallow rock bottom, and is very common among the drifting algae both in Pernambuco and Fernando. W. I., Bermuda to Florida.

DICTYOTA CILIOLATA Kütz. Washed ashore on Pernambuco. W. I., Bermuda to Florida.

DICTYOTA DIVARICATA Lamour. Drifted ashore in Pernambuco. Reported here for the first time from northern Brazil. W. I., Bermuda to Florida.

DICTYOPTERIS ARESCHOUGII (J. Ag.) Vick. Thriving in large quantities on rocky ocean slopes of the reef about three feet below low tide level. Occasionally found drifting ashore both in Pernambuco and Fernando. Endemic to Brazil.

DICTYOPTERIS DELICATULA Lamour. Common in the small pools left in the reef when the tide recedes and on silt in the reef-formed lagoon at low tide level. In Pernambuco and Fernando this species is commonly entangled with other algae drifting near shore. W. I., to Bermuda and Florida.

DICTYOPTERIS PLAGIOGRAMMA (Mont.) Vick. Only found on Fernando where a few specimens were washed ashore. The plants here have a very stout axial stalk to which is attached wiry branches. The midrib is very pronounced and is hairy on one side. In addition to the midrib there are many smaller veins that are pinnately arranged from the midrib. W. I. to Bermuda and Florida.

DICTYOPTERIS JUSTII Lamour. This species grows with *D. Areschougii* and may be distinguished from it in living condition by its darker color and smaller habit, and by the presence of a midrib running to the holdfast. In *D. Areschougii* there is always a short stipe present. W. I., Bahamas, Florida.

PADINA GYMNOSPORA (Kütz.) Vick. In Pernambuco this alga forms large beds growing on silt between the reef and the beach. The plants are light brown and the rows of spores alternate with two rows of hairs. Reported here for the first time from northern Brazil. Caribbean, Gulf of Mexico to Bermuda.

PADINA VICKERSIAE Hoyt. This brown alga and the preceding species share the same habitat here, but this one is more rigidly constructed and is usually darker in color. Caribbean area to North Carolina.

SPATOGLOSSUM ARESCHOUGII J. Ag. This large brown alga shares its habitat with *Dictyopteris Areschougii* in protected places about 3 feet below low tide level on the ocean slopes of the reef. Some specimens measure 22 cm. long and 4 cm. broad below the branches. Endemic to Brazil.

SPATOGLOSSUM SCHRÖEDERI (Mert.) J. Ag. Much smaller than the preceding species and with a dentate margin. Attached to rocks below tide level on Fernando and common among the drifting seaweeds both in Pernambuco and Fernando. W. I. to North Carolina.

ZONARIA VARIEGATA (Lamour.) C. Ag. Washed ashore in Pernambuco. This species has been reported throughout the marine algal range in Brazil. W. I. to North Carolina.

ZONARIA ZONALIS (Lamour.) Howe. Growing from a rocky slope in about 2 feet of water at low tide on the ocean side of the reef. W. I., Bahamas, Florida.

Fucales—Sargassaceae

SARGASSUM LENDIGERUM (L.) C. Ag. In Pernambuco this species is very common growing from the silty bottom between the reef and the beach, where its tips are exposed during low tide. W. I., Bahamas, Ascension Island.

SARGASSUM PLATYCARPUM Mont. In Pernambuco, thriving in beds between the reef and the beach below low tide level where it grows from a rock bottom. W. I. to Bermuda.

SARGASSUM POLYCERATIUM Mont. One of the most common algae on the crest of the reef, exposed at low tide between waves. W. I., Bahamas, Florida.

RHODOPHYCEAE
Bangiales—Bangiaceae

ERYTHROCLADIA SUBINTEGRA Rosenv. Epiphytic on several green algae. W. I., Bermuda, Florida.

Nemalionales—Helminthocladiaceae

NEMALION SCHRAMMII (Crouan) Børg. Growing attached to rock bottom about 6 feet below low tide level between two parallel-arranged reefs. The plants were pink when collected, but became yellowish-brown on drying. Some of the branches attain a length of 21 cm. and range from 2–15 mm. in diameter and are irregularly branched. Study of the preserved material showed both antheridia and carpospores present. The medullary filaments are 6–10 μ in diameter, composed of long rectangular cells without intercellular spaces. The assimilative moniliform filaments are more or less dichotomously branched and consist of oval cells 8–15 μ in diameter, and 15–28 μ long. This is the first report of this species from Brazil. W. I.

ACROCHAETIUM COMPTUM Børg. Living endophytically in the preceding species. This is the first report of this species from Brazil. W. I.

LIAGORA CERANOIDES Lamour. Growing attached in a variety of habitats below low tide level on both sides of the reef. This is the first time a definite station has been established for this species in Brazil. W. I., Bahamas, Florida.

LIAGORA VALIDA Harv. Washed ashore in Pernambuco. Reported here for the first time from Brazil. Guadeloupe Island, W. I., Bermuda, Florida.

Chaetangiaceae

GALAXAURA CYLINDRICA (Sol.) Lamour.[3] This calcified alga was abundant

[3] Because of the structural dimorphism in *Galaxaura* discovered by Howe (1917, 1918) and corroborated by Svedelius (1944) some of the species listed here may eventually be reduced when their life histories are better known.

among algae washed ashore both on Fernando and Pernambuco. W. I., Bermuda, Florida.

GALAXAURA MARGINATA (Ell. & Sol.) Lamour. Drifted ashore on Fernando. Barbados, through W. I., Bermuda and Florida.

GALAXAURA OBLONGATA (Ell. & Sol.) Lamour. Drifted ashore on Fernando. W. I., Bahamas, Bermuda, Florida.

GALAXAURA RUGOSA (Ell. & Sol.) Lamour. One specimen found among drifting plants on Fernando has been assigned to this species. W. I., Bahamas, Bermuda, Florida.

GALAXAURA SUVERTICILLATA Kjellman. In Pernambuco this species was rarely found drifted ashore. This is the first report of this species from Brazil. W. I., Bahamas, Bermuda, Florida.

Gelidiales—Gelidiaceae

GELIDIUM RIGIDUM (Vahl.) Grev. This is the most common littoral alga at Pernambuco and Fernando. On the reef it grows in a variety of habitats where it is frequently interwoven with other algae. W. I., Bermuda, Bahamas, Florida.

GELIDIUM CCRNEUM (Huds.) Lamour. This species forms dark, tough tufts where it grows at low tide level on the reef. W. I., Bahamas, Florida.

GELIDIUM CRINALE (Turn.) Lamour. Found growing with *Laurencia papillosa* at low tide level on the reef. Maine to Florida, and W. I.

GELIDIUM PUSILLUM (Stack.) LeJolis. Firmly attached to surface of cliff and sprayed at low tide on Fernando. Distribution not well known.

GELIDIUM sp. A light red alga, sparingly branched which in cross section shows the structure of this genus. It has not been possible so far to assign this satisfactorily to any described species.

Cryptonemiales—Corallinaceae

FOSLIELLA LEJOLISII (Rosanoff) Howe. Epiphytic on various species of algae in Pernambuco and Fernando. Northeastern U. S. to Brazil.

MELOBESIA MEMBRANACEA (Esper) Lamour. Epiphytic on various species of algae in Pernambuco and Fernando, often mixed with the preceding species. This is the first report of this species from Brazil. W. I. to Florida.

JANIA ADHAERENS Lamour. This tiny plant is epiphytic on *Sargassum polyceratium* in Pernambuco. W. I., Bahamas, Florida.

JANIA CAPILLACEA Harv. One large tuft washed ashore south of Recife. W. I., Bahamas, Florida, and North Carolina.

JANIA PUMILA Lamour. Epiphytic on *Dictyopteris Areschougii* and *Sargassum polyceratium* from the reef. First report from Brazil. W. I., Bahamas, Bermuda and Florida.

JANIA RUBENS (L.) Lamour. Washed ashore with an entanglement of *Amphiroa fragilissima* and *Dictyopteris delicatula* on reef off Pernambuco. W. I., Bahamas, Bermuda, Florida.

AMPHIROA BEAUVOISII Lamour. Growing in tufts in depressions in the reef. It is uncommon on the reef, but common among the algae washed ashore. This species has a resemblance to *A. rigida* Lamour. var. *antillana* Børg. Distribution not well known.

AMPHIROA FRAGILISSIMA (L.) Lamour. One tuft mixed with the preceding species was found growing in a depression on the reef below low tide level. Fragments of this species were plentiful on the beach. W. I., Bermuda, to North Carolina.

CORALLINA CUBENSIS (Mont.) Kütz. emend. Børg. A very common epiphyte on *Digenia simplex* off Pernambuco. W. I., Bahamas to Florida.

Grateloupiaceae

CRYPTONEMIA CRENULATA J. Ag. Four specimens found washed ashore in Pernambuco. W. I., Florida to North Carolina.

CRYPTONEMIA LUXURIANS (Mert.) J. Ag. Two plants washed ashore in Pernambuco. W. I., Bermuda to Florida.

HALYMENIA FLORESIA (Clem.) C. Ag. Common on rock bottom about 6 feet below low tide level in lagoon between parallel arranged reefs. W. I., Florida to North Carolina.

HALYMENIA FLORIDANA J. Ag. Growing with the preceding species but less common. Reported here for the first time from Brazil. Ranges as far north as North Carolina.

Gigartinales—Nemastomataceae

PLATOMA TENUIS Howe & Taylor. About a dozen small plants found washed ashore in Pernambuco. Caribbean area.

Rhodophyllidaceae

RHODOPHYLLIS GRACILARIOIDES Howe & Taylor. Attached to ocean slopes of reef below low tide level. Known only from Brazil. First report from northern Brazil.

Rhabdoniaceae

RHABDONIA RAMOSISSIMA (Harv.) J. Ag. var. DILATATA J. Ag. Two plants found drifted ashore in Pernambuco. First report from Brazil. W. I., to Florida.

GYMNOGONRGUS sp. A few plants drifted ashore in Pernambuco. On the basis of form and structure this alga undoubtedly belongs to this genus. The plants lack reproductive structures. It is thicker and tougher than *G. tenuis* J. Ag. from the W. I.

Hypneaceae

HYPNEA CERVICORNIS J. Ag. One large specimen found drifting off Pernambuco. Reported here for the first time from northern Brazil. W. I., Bermuda to Florida.

HYPNEA MUSCIFORMIS (Wulf.) Lamour. This very variable alga becomes a coarse plant in Pernambuco, with numerous spiny projections. It grows in large beds on a rocky bottom just below low tide levels in the reef-formed

lagoon. It is very abundant in Brazil. It is an excellent source of Hypnea (agar) jel. Brazil to Massachusetts.

HYPNEA SPINELLA (C. Ag.) Kütz. This is a very common alga growing in mats and sometimes mixed with *Bostrychia Binderi* on the ocean wall of the reef and on cliffs hit by spray at low tide at Fernando. W. I. to Bermuda.

HYPNEA ESPERI Bory. A wiry, spiny tough small Hypnea growing entangled with *Botryocladia Uvaria* on the reef. Chile, Easter Islands.

Gracilariaceae

GRACILARIA BLODGETTII Harv. Growing below low tide levels on rock bottom near the beach in a lagoon formed by the reef. First report here for Brazil. Virgin Islands to Florida.

GRACILARIA CERVICORNIS (L.) Grev. Growing in very turbulent water on ocean slopes of the reef where it is exposed to intermittent pounding waves during low tide. One of the most common algae of the reef. Also found free floating off Fernando. W. I., Bermuda and Florida.

GRACILARIA CORNEA J. Ag. Growing on the ocean side ledge of the reef where it receives a pounding from waves at low tide; also common between the reef and the beach on rocky bottom. In this latter habitat it is a more slender plant than the stubby form of the ledge. Reported only from this station in Brazil. W. I., Bahamas, to Florida.

GRACILARIA CYLINDRICA Børg. Very common on the rock bottom in the lagoon between the reef and the beach. W. I. to Florida.

GRACILARIA CURTISSIAE J. Ag. Growing on the ocean ledge of the reef, and in the lagoon between the reef and the beach where it grows attached to rock bottom. Florida.

GRACILARIA FEROX J. Ag. Thriving on top of the reef in turbulent water, where it is exposed between waves at low tide. W. I., Bermuda to Florida.

GRACILARIA MAMMILLARIS (Mont.) Howe. In Pernambuco forming a part of the carpet with other algae on top of the reef, where it is exposed at low tide. This is the first report of this species from northern Brazil. Guadaloupe to North Carolina.

GRACILARIA ORNATA Aresch. One of the toughest of the algae of the ledges on the ocean side of reef, pounded by waves during low tide. Known only from this station in Brazil.

Rhodymeniales—Rhodymeniaceae

BOTRYOCLADIA UVARIA (Wulf.) Kylin. This olive-brown algae grows in profusion on the ocean side slopes of the reef in the intertidal zone, where it is exposed to pounding waves at low tide. The Brazilian specimens differ from those of the West Indies in that the bladders are about half as large and are essentially sessile. W. I., Florida and North Carolina.

BOTRYOCLADIA SKOTTSBERGII Børg. (Figure 3.) Common on the inner walls and the transverse fissures of the reef intermittently exposed during low tide. A sample of this plant has been submitted to Dr. Børgesen who states that it superficially resembles his described species (1924) but he

reserved a final report pending a microscopic examination. After a study of the original description and herbarium specimens of related species, we have placed it in this species. The type locality is Easter Islands, where it was endemic until this report.

Champiaceae

CHAMPIA PARVULA (C. Ag.) Harv. Epiphytic on other algae about the reef. W. I., east coast of North America as far north as New England.

Ceramiales—Ceramiaceae

CENTROCERAS CLAVULATUM (C. Ag.) Mont. Epiphytic on *Digenia simplex* and growing in tufts on the top of the reef. Also epiphytic on various algae from Pernambuco and Fernando. Tropical Western Atlantic.

CERAMIUM NITENS (C. Ag.) J. Ag. Frequently encountered entangled with other algae of the reef off Pernambuco. First reported here from Brazil. W. I., to Florida.

CERAMIUM RUBRUM (Huds.) C. Ag. A common epiphyte on several algae, especially *Bostrychia Binderi* from both Pernambuco and Fernando. W. I. and Atlantic coast of North America.

GRIFFITHSIA GLOBULIFERA (Harv.) J. Ag. Free floating and mixed with *Dictyopteris delicatula* in Pernambuco. Reported for the first time from Brazil. W. I., and Atlantic coast of North America.

GRIFFITHSIA RADICANS Kütz. One large sterile plant found drifting between the reef and the beach. General distribution unknown.

SPYRIDIA FILAMENTOSA (Wulf.) Harv. One large plant found washed ashore in Pernambuco. W. I. and Atlantic coast of North America.

Rhodomelaceae

LAURENCIA OBTUSA (Huds.) Lamour. One of the carpet algae on top of the reef, exposed at low tide. W. I. to Florida.

LAURENCIA PAPILLOSA (Forssk.) Grev. One of the dominant species on top of the reef, exposed during low tide. W. I. to Florida.

LAURENCIA SCOPARIA J. Ag. Growing with other algae on top of the reef. Bermuda.

CHRONDRIA LITTORALIS Harv. Growing in silt at the base of shore-facing wall of the reef below low tide level. Reported here for the first time from Brazil. W. I. to east coast of the United States.

DIGENEA SIMPLEX (Wulf.) C. Ag. From various habitats in turbulent waters from Pernambuco and Fernando. Usually covered with epiphytic *Centroceras* and *Corallina*. W. I., Bermuda, Bahamas, to Florida.

ACANTHOPHORA SPICIFERA (Vahl.) Børg. This is one of the dominant algae on ledges of the ocean side of the reef, where it is exposed to turbulent waters especially at low tide. W. I., Bahamas, Bermuda to Florida.

BRYOTHAMNION SEAFORTHII (Turner) Kütz. f. DISTICHA J. Ag. A co-dominant alga with *Acanthophora spicifera*. W. I. to Florida.

BRYOTHAMNION TRIQUETRUM (Grelin) Howe. Growing attached to rocks in very turbulent water, exposed intermittently at low tide on Fernando. W. I., Bahamas to Florida.

BOSTRYCHIA BINDERI Harv. Forming mats on vertical ocean walls of reef off Pernambuco and on cliffs at Fernando. Sprayed or lashed by waves during low tides. W. I. to Florida.

BOSTRYCHIA SERTULARIA (Mont.) Howe. On ocean wall of reef, exposed at low tide. Grows in less turbulent places than the preceding species. Bermuda and Bahamas.

BOSTRYCHIA TENELLA (Vahl) J. Ag. One specimen found drifting between the beach and the reef. First report of this species from northern Brazil. W. I., Bahamas, Bermuda to Florida.

HERPOSIPHONIA TENELLA (C. Ag.) Ambronn. Washed ashore on Pernambuco. This is the first report of this species from Brazil. W. I., Bahamas, Florida to North Carolina.

LOPHOSIPHONIA OBSCURA Auct. Commonly found growing interwoven with *Bostrychia Binderi* on ocean walls sprayed at low tide, Pernambuco and Fernando. Reported here for the first time from Brazil. W. I. to Florida.

AMANSIA MULTIFIDA Lamour. 'One of the members of the algal carpet on the crest of the reef, exposed at low tide. W. I. to Florida.

VIDALIA OBTUSILOBA (Martens) J. Ag. A tough, dark alga and one of the dominant algae of the ocean side ledges of the reef, where it is exposed to turbulent water at low tides. W. I. to Florida.

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EXTENSION OF THE BROWN ALGAL ORDER DICTYOSIPHONALES TO INCLUDE THE PUNCTARIALES

GEORGE F. PAPENFUSS

In 1933 Professor Kylin published a paper entitled "Über die Entwicklungsgeschichte der Phaeophyceen," which formed a landmark in the advancement of knowledge of the brown algae. In addition to contributing much new information on the life histories of some eighteen species, he utilized the available knowledge of the structure and reproduction of the brown algae to build a new system of classification.

Kylin divides the Phaeophycophyta into three classes and twelve orders. The first class, the Isogeneratae, includes the Ectocarpales[1] (Setchell & Gardner 1922), Sphacelariales (Oltmanns 1922), Cutleriales (Oltmanns 1922), Tilopteridales (Kylin 1917), and Dictyotales (Kjellman 1893). The second class, the Heterogeneratae, includes two subclasses, the Haplostichineae and Polystichineae. To the first of these belong the Chordariales (Setchell & Gardner 1925), Sporochnales (Sauvageau 1926), and Desmarestiales (Setchell & Gardner 1925), and to the second the Punctariales (Kylin 1933), Dictyosiphonales (Setchell & Gardner 1925), and Laminariales (Kylin 1917). The third class, the Cyclosporeae, includes the single order Fucales (Kylin 1917).

Although there was, and still is, uncertainty as to the ordinal position of some of the genera or families, the arrangement of Kylin has met with approval and has been followed by almost all phycologists. One notable exception, however, is Fritsch (1945). He does not accept the orders Chordariales, Punctariales, and Dictyosiphonales, but retains the families composing them in their former position in the order Ectocarpales (cf. Oltmanns 1922). Although agreeing to the separation of the Sporochnaceae and Desmarestiaceae from the Ectocarpales and the establishment of autonomous orders for them, the order Ectocarpales *sensu* Fritsch still includes

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[1] An order Ectocarpales was established in 1922 independently by Setchell and Gardner (1922) and Oltmanns (1922). The date of publication of the article by Setchell and Gardner is May 16, but the exact date of appearance of the volume by Oltmanns is not indicated. It seems certain, however, that it was published later than the article by Setchell and Gardner. In volume 44 (1922) of R. Friedländer und Sohn's *Naturae Novitates,* reference to the work by Oltmanns appears for the first time in the November (No. 11) issue (p. 244), whereas reference to the paper by Setchell and Gardner (which was published in the United States of America) appears in the August–September (Nos. 8 & 9) issue (p. 192).

an assortment of algae almost as heterogeneous, in regard both to structure and life history, as it did in the system of Oltmanns (1922).

In the past the classification of the higher categories of brown algae was largely based on structure. Although structure is still of great importance in the separation of related species, genera, families, and, in certain instances, even orders, the system of Kylin offered a notable departure in that it was to a large extent founded upon life histories. In view of the transcendent value of the type of life history as an indicator of phylogenetic relationship in lower organisms, the arrangement of Kylin must be regarded as portraying more accurately the basic relationships among brown algae than do earlier systems.

Much of our knowledge of the life histories of brown algae has been acquired in comparatively recent times. This information was not forthcoming until it had been shown that the complete cycle of life could not be learned unless the plants were grown in culture. To Sauvageau, more than to any other person, we are indebted for leadership in this approach to problems of this kind. Over a period of about thirty years previous to his death in 1936, he contributed a long series of papers dealing with the life histories of Phaeophycophyta. (For a list of his publications, see Dangeard 1937.) The discovery for which he probably is best known to botanists in general is that of the microscopic gametophytic generation of members of the Laminariales in 1915 and 1916.

In 1917 Sauvageau announced the discovery of an alternation of heteromorphic generations in *Dictyosiphon foeniculaceus*. Comprehending the full significance of this, Setchell and Gardner in 1925 utilized this character and that of the method of growth of the sporophytic thallus by means of a single apical cell as criteria for removing *Dictyosiphon* from the Ectocarpales and establishing for it the order Dictyosiphonales.

A family Dictyosiphonaceae had already in 1890 been erected by Kjellman for *Dictyosiphon* and *Gobia*, and in 1895 De Toni had added *Scytothamnus* to it. Setchell and Gardner were of the opinion that *Gobia* and *Scytothamnus* more properly belonged in their newly established order Chordariales. *Scytothamnus*, at least as to its type species *S. australis*, has been shown by Kuckuck (1930) and others to possess a subterminal meristem and it was recently placed by Levring (1941) in the family Chnoosporaceae of the order Punctariales, which is probably the correct place for it. *Gobia*, on the other hand, has been shown by Du Rietz (1940) and Levring (1940), working independently of each other, to be synonymous with *Dictyosiphon*.

Since the time of De Toni, Oltmanns (1922), Kuckuck (1930), and Fritsch (1945) have added one or more of the genera *Coilodesme*, *Delamarea*, and *Cladothele* to the Dictyosiphonaceae. The systematic position of these genera is, however, still uncertain and some authors have placed them in other

families. Although in general structure they agree with *Dictyosiphon*, the method of growth by a single apical cell has thus far been shown to occur in *Cladothele* only (Skottsberg 1921, fig. 15a). According to Reinke (1889–1892), very young plants of *Coilodesme* might possess an apical cell. In later stages, however, he and Kuckuck (1930) both found growth to occur as the result of intercalary cell divisions. Reinke (op. cit., p. 52) says: "Vielleicht wird man finden, das Keimpflanzen von *Coilodesme* eine Scheitelzelle besitzen, aber auch wenn das nicht der Fall sein sollte, würde ich wegen der eigenthümlichen Entwicklung der sporangientragenden Gewebeschichten kein bedenken tragen, *Coilodesme* zu der Dictyosiphoneae zu stellen."

The Dictyosiphonales were placed by Kylin (1933), together with the Laminariales and his newly erected Punctariales, in the subclass Polystichineae of the class Heterogeneratae. A distinguishing feature of the Polystichineae is the formation of true parenchymatous tissues as the result of intercalary longitudinal division of the cells of the thallus.

The justification for recognition of an order Laminariales has, to the knowledge of the writer, not been questioned since its establishment by Kylin in 1917. Whether the Dictyosiphonales and Punctariales ought to be kept apart is, however, less certain. The only feature whereby they are separated is that in the Dictyosiphonales the thallus grows by means of a single initial, whereas in the Punctariales growth is subapical (e.g. *Chnoospora*) or diffuse. However, even in those Dictyosiphonales, such as *Dictyosiphon foeniculaceus*, in which the apical cell apparently remains functional, intercalary transverse divisions are not uncommon and no doubt play an important role in the growth of the thallus. In others, such as *Gobia baltica*, which is synonymous with *Dictyosiphon Chordaria*, young individuals grow by means of a terminal cell, but in older stages the apical cell is functionless and growth is intercalary. Reinke (1889–1892, p. 52) says of this species: ". . . *Gobia* zeigt nur an ganz jungen Individuen ein Wachsthum mit Scheitelzelle, später wächst es ganz intercalar ohne Scheitelzelle, wie auch *Dictyosiphon Chordaria* in seinen älteren Zuständen." In *Cladothele Decaisni* the situation is much as in *D. Chordaria*. Skottsberg (1921, p. 36) remarks of this species: "In *Cladothele* every branch ends in an apical cell (fig. 15a). . . . the apical cell is not an initial in the true sense, but becomes less active at an early date, . . ."

In the opinion of the writer it thus seems best to unite the Punctariales and Dictyosiphonales under the latter name, which has the right of priority. Further support for this view, although indirect, is furnished by the situation in the Chordariales. Here the members of some families grow by means of a single initial cell, whereas in others growth is by an intercalary (trichothallic) meristem.

As thus extended the order Dictyosiphonales would include the families: Striariaceae (Kjellman 1890), Giraudyaceae (Sauvageau 1927), Myriotrichiaceae (Kjellman 1890), Punctariaceae (Kjellman 1883), Chnoosporaceae (Setchell & Gardner 1925), Dictyosiphonaceae (Kjellman 1890).

It is not unlikely that the Punctariaceae (Encoeliaceae of some authors) should be divided into several families as, in fact, has been done by Setchell and Gardner (1925) and others. However, until this can be done along more sharply defined lines than is at present possible, it may be best to recognize but one family.

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A BASIDIOBOLUS PRODUCING ELONGATED SECONDARY CONIDIA WITH ADHESIVE BEAKS

CHARLES DRECHSLER[1]

A maizemeal-agar plate culture which after being permeated with *Pythium ultimum* Trow had been further planted with small quantities of friable leaf mold collected on November 14, 1945, in a deciduous wood near Mercer, Wisconsin, showed after 50 days about a hundred globose conidia scattered sparsely over an area of approximately 75 square millimeters bordering one of the deposits of forest detritus. Nearly all of the globose bodies were empty of protoplasmic contents, and thus were represented only by membranous envelopes many of which had become misshapen from partial collapse (fig. 1, a). The globose envelopes that had suffered no collapse (figs. 2, a; 3, a; 4, a) revealed, like the few globose conidia that retained their protoplasmic contents, a rather broadly protuberant irregularity in their rounded contour, which gave them an unmistakable similarity to the subspherical conidia characteristic of many insectivorous Entomophthoraceae as well as of the few related forms in the genera *Conidiobolus* and *Delacroixia*. Many of the empty globose envelopes were found bearing individually an erect slender conidiophore (figs. 1, b; 2, b; 3, b; 4, b) usually about 300 μ high, on whose tip was supported an elongated ellipsoidal or strobiliform secondary conidium (figs. 1, c; 2, c; 3, c) prolonged at its distal end into a narrow beak which terminated in a relatively large spherical mass of yellow material. While in many instances the empty conidiophore was found denuded (fig. 4, b), and often, besides, had fallen over prostrate on the substratum, it was clear that all of the empty globose conidia had used their contents in producing aloft a secondary conidium of the beaked strobiliform type. In no instance had germination taken place by the production of a mycelial hypha.

Nor was vegetative germination observable in any of the beaked conidia. When these fell on the moist substratum (figs. 5, a; 6, a; 7, a; 8, a) they usually would send up a slender conidiophore (figs. 5, b; 6, b; 7, b; 8, b), most frequently from a position about midway between base and apex, to produce a tertiary conidium (fig. 5, c), which, like its immediate parent, was strobiliform and drawn out distally into a beak surmounted by a subspherical yellowish mass. The same reproductive development would commonly be

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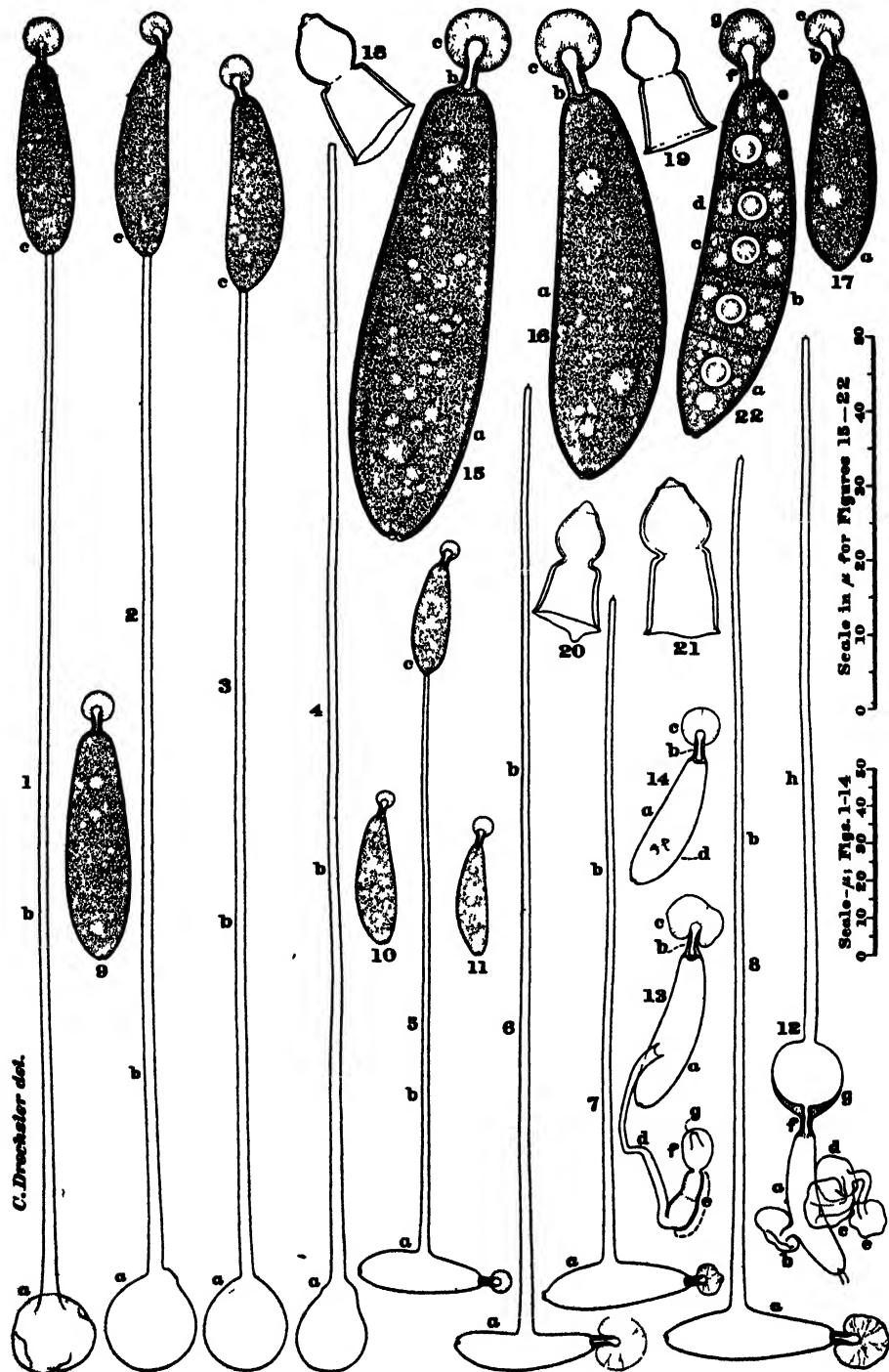
repeated a second and a third time, each repetition entailing some reduction
in size of the spore; so that whereas the first generation of strobiliform
conidia often measured 64 to 73 μ in total length and 14.5 to 17.5 μ in greatest
width (figs. 1, c; 2, c; 3, c; 9), many of their descendants (figs. 5, c; 10; 11)
showed corresponding dimensions only half as large.

Departures from the usual course of repetitional development were mani-
fest in a number of instances. In one such instance the empty spore envelope
of elongate elliptical outline (fig. 12, a) bore in its median region four empty
collapsed membranous pouches, one of them being attached by a short hyphal
outgrowth (fig. 12, b), the others (fig. 12, c–e) being borne at intervals on a
hyphal outgrowth that must have been produced by successive elongation.
That these collapsed pouches may originally have had a subspherical shape
was strongly indicated in the distal modification of the beak (fig. 12, f) into
a globose inflated part (fig. 12, g) that bore a slender sporophore (fig. 12, h)
whereon had evidently been produced a conidium of the strobiliform type.
The inflated part at the tip of the beak would seem perhaps best interpreta-
ble as a somewhat abortive conidium of the globose type interpolated between
two generations of strobiliform conidia. The four collapsed membranous

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Explanation of figures 1–22

Basidiobolus haptocladus, drawn with the aid of a camera lucida; magnification
× 500 in figures 1–14, and × 1000 in figures 15–22. FIGS. 1–3. Globose primary conidia,
a, each of which has put forth an erect conidiophore, b, whereon is borne an elongate
secondary conidium, c, with its apical beak supporting a mass of yellow adhesive mate-
rial. FIG. 4. Empty envelope of a globose conidium, a, with the empty membrane of
the erect germ conidiophore, b, from which an elongate conidium has become detached.
FIG. 5. Elongate conidium, a, which has put forth an erect conidiophore, b, and on its
tip has produced an elongate beaked daughter-conidium, c. FIGS. 6–8. Empty envelopes
of elongate conidia, a, each bearing an empty erect conidiophore, b, from whose tip a
daughter-conidium of elongate type has become detached. FIGS. 9–11. Detached conidia
of elongated beaked type, showing differences in size and shape. FIG. 12. Empty envelope
of elongate conidium, a, bearing laterally on two short empty hyphal outgrowths the col-
lapsed membranous envelopes of four globose bodies, b–e, and bearing distally on its
apical beak, f, a fifth empty globose body, g, whereon is supported an empty erect
conidiophore, h, now denuded of the elongate daughter-conidium produced on its tip.
FIG. 13. Empty envelope of elongated conidium, a, with terminal beak, b, and flattened
body of adhesive material, c; it bears laterally a germ conidiophore, d, which tapers nar-
rowly before widening out to bear an abortive "basidium," e, surmounted by the empty
envelope of an abortive globose conidium, f, supporting a sterigma, g, from which an
elongate conidium was disjointed. FIG. 14. Empty envelope of elongate conidium, a,
with apical beak, b, and mass of adhesive material, c; it bears laterally a short, distally
reflexed, empty, tubular membrane, d, left behind after the violent discharge of an ex-
panded "basidium" and globose conidium. FIGS. 15–17. Elongate conidia, a, each with
an apical beak, b, whereon is borne a globose mass of yellow adhesive material, c. FIGS.
18–21. Empty membranous remains of expanded distal parts of propulsive conidiophores
("basidia") found lying scattered on substratum after each functioned in the violent
discharge of a globose conidium. FIG. 22. Elongate conidium which has become divided
internally into five cells or sporangiospores, a–e, each showing a large nucleus near its
center; f, apical beak; g, globose mass of yellow adhesive material.

pouches seem likewise interpretable as abortive globose conidia that with respect to function had suffered complete frustration.

Another and somewhat different departure from the usual course of repetitional development was brought to light in a strobiliform conidium whose empty envelope (fig. 13, a), correctly provided at its apex with beak (fig. 13, b) and mass of yellowish material (fig. 13, c), was found bearing in the usual median position an empty conidiophorous filament (fig. 13, d), which at a distance of 35 μ from its origin, on tapering to a width of 1.3 μ, widened out to 2.5 μ and maintained this width for a distance of 20 μ to bear a thick-walled expanded part (fig. 13, e) surmounted by a thin-walled, somewhat globose, empty bladder (fig. 13, f). This bladder bore an empty, blunt, sterigma-like projection (fig. 13, g) from which assuredly a daughter-conidium must have become abjointed. Judging from the narrow apex of the projection, the daughter-conidium most probably was of the beaked strobiliform type. Here, again, the globose bladder may best be construed as a poorly developed abortive conidium of the subspherical type that was interpolated between two generations of beaked conidia.

That, nevertheless, a conidium of the strobiliform type may sometimes give rise successfully to a daughter-conidium of the globose type was inferred from an empty spore envelope (fig. 14, a) which showed a fully characteristic elongated-elliptical outline and at the apex was prolonged into a beak (fig. 14, b) bearing a subspherical mass of yellow material (fig. 14, c). The only membranous part present on this envelope that could have been operative in conveying away the protoplasmic contents was a short lateral tube (fig. 14, d), open at the end, and with its distal portion markedly widened and reflexed; the cylindrical portion of the tube measuring about 2.5 μ in length and width, while its reflexed mouth showed a width of approximately 5.5 μ. As the slightly lipped basal hilum in elongated beaked conidia (figs. 15, a; 16, a; 17, a), with its darkened minute central scar, is usually not more than 2 μ wide, the likelihood that a conidium of this type could have been disjointed from so broad an attachment can be dismissed from consideration. The manner in which the reflexed tube must have come into being was made evident through the presence near the leaf mold of more than twenty thick-walled empty structures (figs. 18–21) consisting individually of a bell-shaped part, slightly flaring at the open mouth, together with a slightly shorter dome-shaped part that at the closed end showed a minute thin-walled protrusion; the two parts being demarcated from one another by a fairly pronounced transverse constriction. The close resemblance of these curious structures to the "basidia" figured by Eidam (10, *pl. 10, fig. 22, a–c*) in the original account of his *Basidiobolus ranarum,* as well as to Thaxter's (20, *pl. 21, figs. 411, a; 412, a*) illustrations of the same objects, identified them unmistakably as thick-walled envelopes of the expanded ter-

minal parts thrown off in the violent·discharge of conidiophores pertaining to a species of *Basidiobolus*. Manifestly the reflexed tube on the empty strobiliform conidial envelope represented the proximal portion of such a propulsive conidiophore that was left attached when the expanded distal portion, together with the daughter-conidium borne on it, was projected into the air. The conidiophore here must have been very short since the expanded part had evidently been borne on a narrow stalk only 2.5 μ long. Further, the meager length of the reflexed rim of the empty tube would seem to indicate that rupture of the conidiophore here took place considerably farther toward the base of the expanded part than would seem illustrated by Ingold (13) for *B. ranarum*.

There is reason to believe that in the present fungus the narrower stalk-like part of the propulsive conidiophore may often attain greater length than in the specimen shown in figure 14, d. As the thick-walled expanded part shown in figure 13, e, almost certainly represents an abortive "basidium," the somewhat widened portion of supporting filament immediately below it may very probably have developed as the stalk of the "basidium," whereas the narrowly ·tapering proximal portion of supporting filament offered more the appearance of having been produced as the prospective stalk of an elongated beaked conidium. Direct observations on the forcible discharge of the "basidium" and globose conidium are lacking, because unfortunately the projection of these bodies on to the transparent agar, evidently from a source some distance within the deposit of leaf mold, had come to an end when the fungus was first seen; and because, further, the repetitional development of conidia on the agar took place, with very few exceptions, by the production of elongated beaked conidia on inert slender sporophores.

Spores corresponding to the beaked conidia of the Wisconsin fungus do not seem to have been ascribed to *Basidiobolus ranarum* in the rather extensive literature on that species. Nor apparently do any references to such spores occur in the less abundant writings on *B. lacertae* Eidam (10, 15), which species Levisohn (14), after ample comparative study carried out with excrement of frogs, toads, salamanders, blindworms, and lizards, concluded should be reduced to synonymy with *B. ranarum*. Mention of elongated beaked conidia likewise seems absent in Fries' original account of *B. myxophilus* (11), a species which its author (12) later seemed inclined to regard as also being identical with *B. ranarum*, on learning that the gelatinous masses in which it developed might have consisted of material voided from the oviducts of frogs. Of problematical import in this connection is a figure of *B. ranarum* given by Eidam (10, *pl. 9, fig. 16*) wherein is shown a globose conidium with a long slender erect germ-conidiophore that supports an expanded structure tapering markedly toward a narrow apex on which is attached a small globose body. The expanded structure was construed by

Eidam as a "basidium" that had begun to bud forth a tertiary conidium. Thaxter (20, p. 145, 146) noted its similarity to the nearly almond-shaped secondary conidia often produced by *Empusa sphaerosperma* Fres., *E. Fresenii* Nowak., and allied insectivorous species. These secondary conidia Thaxter found borne on long slender capillary conidiophores from which they were not forcibly discharged; and he observed them to germinate by means of an irregular hypha that begins "as a drop-like protuberance from the apex of the spore." One of his figures of *E. Fresenii* (20, *pl. 16, fig. 119*) shows a globose primary conidial envelope that has become emptied of its contents in sending up a capillary conidiophore on whose empty tubular membrane is supported aloft an almond-shaped secondary conidium, which, while still attached, has begun to germinate by putting forth a small spherical protuberance at its tip. This figure, as also Eidam's figure referred to, reveals with respect to the outward shape and general arrangement of component parts a suggestive approximation to the asexual reproductive units of the Wisconsin fungus in which a secondary elongate beaked conidium has been produced from a globose primary conidium.

Although the elongate beaked conidia here under discussion appear truly homologous with the almond-shaped conidia of *Empusa Fresenii*, they show some distinctive differences. As far as I have been able to determine they are always terete, never being flattened laterally in any noticeable degree. The beak (figs. 15, b; 16, b; 17, b; 22, f) with which they are regularly provided cannot well be regarded as a product of incipient vegetative germination, seeming rather to represent a product of spore differentiation. In mature conidia it consists of a sturdy narrowed cylindrical prolongation of the spore envelope. It commonly ranges in length from 4 to 8 μ, and in width from 1.5 to 2.5 μ. Its wall is distinctly yellow and except at the distal end has usually a thickness of 0.5–1 μ. The empty and frequently narrow lumen of the beak is separated from the protoplasmic interior of the living cell below by a septum which likewise is thick and distinctly yellow. Distally the tubular wall diminishes pronouncedly in thickness, so that at the very tip it may be discernible only as a thin convex line of demarcation setting off the colorless lumen from the homogeneous globose mass of yellow material, 5–10 μ in diameter, that surrounds the terminal portion of the beak (figs. 15, c; 16, c; 17, c). In some instances the subspherical mass may show at its periphery a perceptibly different shade of coloration, either lighter (fig. 22, g) or deeper than through its interior. After the spore has fallen on the substratum, the mass often flattens out somewhat irregularly and may then show a variable number of radial striations (figs. 6, a; 7, a; 8, a; 13, c; 14, c). These striations strongly recall the radial markings frequently observable in the globose masses of adhesive material utilized by my *Nematoctonus haptocladus* (9) in the capture of nematodes, and very probably likewise merely represent

minute folds formed in a thin film-like outer layer when the mass becomes flattened under external pressure. Parallelism with *N. haptocladus* is further evident in that the yellow material of the Wisconsin fungus is of an adhesive character, and obviously is secreted from the thin-walled distal portion of the beak. Among phycomycetes the glandular conidial beak, together with the globose adhesive mass secreted by it, has an approximate counterpart in my *Stylopage rhynchospora* (4, p. 394–397), where, however, transformation of the conidial beak into a glandular part is more often omitted than accomplished.

Presumably the special function of the adhesive beak is to attach the spore securely to a passing animal. Although a possibility exists that such attachment be serviceable merely in achieving dispersal after the manner of cockleburs (*Xanthium* spp.), analogy with other fungi whose spores are equipped with similar organs while still attached to their sporophores—e.g. *Dactylaria haptospora* Drechsl. (6, p. 456–461) and *Nematoctonus leptosporus* Drechsl. (7)—or whose spores develop comparable adhesive organs after they fall on the substratum—e.g. *Dactylella asthenopaga* Drechsl. (3, p. 496–499) and *Nematoctonus pachysporus* Drechsl. (7)—seems strongly indicative of a parasitic or, perhaps, predaceous relationship. To some degree the fungus would seem to betray a dependence on some other organism also by its failure to grow vegetatively on the agar culture wherein it appeared. In restricting its development to the production of successive generations of spores it showed a behavior much like the behavior that on a few occasions was found displayed by some insectivorous entomophthoraceous forms in similar cultures which had been prepared by adding detritus from herbaceous crop plants evidently containing intermixed remains of parasitized aphids. By way of contrast, when in several instances *Delacroixia coronata* (Cost.) Sacc. & Sydow (1; 19, p. 457) developed from plantings of decaying material, it gave rise to an extensive vegetative mycelium that yielded an abundance of conidia and resting spores.

Among the many elongated beaked conidia that came under observation, two individuals—one of them shown in figure 22—arrested special attention, for instead of giving rise to another spore externally they became divided internally by transverse cross-walls. The five resulting cells (fig. 22, a–e), though not accurately equal in size, showed no great differences in volume. The two subconical or dome-shaped cells at the base and tip (fig. 22, a, e) measured about 13 μ in length, and about 10 or 11 μ in diameter at the circular end directed toward the middle of the spore; while the three intermediate segments (fig. 22, b–d), of cylindrical shape, measured 6.5–8 μ in length and 10–11.5 μ in diameter. Each of the segments contained near the center of its vacuolate protoplast a spherical nucleus, 4–4.5 μ in diameter, which showed a clear hyaline outer layer surrounding a noticeably darker subspherical

nucleolus commonly 2.2–2.5 μ in diameter. Owing to its relatively large size and its ready visibility in an unstained condition within the living cell, the nucleus here had much resemblance to the nucleus of various rhizopods, such as *Amoeba verrucosa* Ehrenb., for example, where similarly a hyaline outer layer surrounds a slightly darker subspherical central body. Eidam remarked on the unusually large size of the nucleus in *Basidiobolus ranarum*, and mentioned being able to see it in unstained living material. He further characterized the nucleolus of his species as being very large, though judging from his figures it would seem to be somewhat smaller in comparison with the entire nucleus than the nucleolus of the Wisconsin fungus. While for the most part, again, Eidam figured the nucleus of *B. ranarum* with an elliptical outline, the nucleus in the conidial segments of the Wisconsin fungus appeared to be very nearly spherical. It seems unlikely, however, that specific differences are concerned here, since in the figures given by Raciborski (18), Olive (17), and Levisohn (14), the nucleus of *B. ranarum* shows considerable variation in shape and in relative size of the nucleolus.

The two segmented conidia were left on the surface of the agar where they had fallen. They were examined at intervals until after 12 days the culture was ruined through burrowing of small annelids. During these 12 days they showed no sign of further change. In becoming divided internally into several cells they evidently developed in accordance with their primitive character as sporangia. The several cells formed within them must be regarded as sporangiospores homologous with the endogenous immotile spores of my *Gonimochaete horridula* (8) as well as with the cells which according to Levisohn's account are liberated from the globose conidial envelopes of *B. ranarum* inside the digestive tubes of frogs and lizards. Moreover, the several cells of the elongated conidium may readily be homologized with the multiple segments of the helicoid hyphal terminations of my *Meristacrum asterospermum* (5), each of which gives rise exogenously, by budding, to a single conidium of rather small size. To the small conidia of *M. asterospermum*, therefore, as also to the similarly small conidia produced plurally on multiple stalks put forth by the large globose conidia of *Delacroixia coronata* and *Conidiobolus Brefeldianus* Couch (2), the multiple spore segments of the Wisconsin fungus would seem to bear the same relationship as the sporangiospores of *Rhizopus* bear to the conidia of *Cunninghamella*.

It is not known whether the leaf mold used in planting my culture contained excrement of any of the animals habitually harboring *Basidiobolus ranarum* in their digestive tracts, for though the material was collected on rolling upland in a site well removed from any pond or permanent stream, some species of frogs and toads roam about so widely that their excrement might be distributed, even if only in meager quantity, almost anywhere. Consequently the source of the detritus provides no certain indication as to

the separateness of my fungus from *B. ranarum;* and, indeed, caution would seem here all the more necessary in view of Möller's report (16, p. 5–8) that several times *B. ranarum* developed spontaneously in decaying plant residues he collected on the side of a forest brook in Brazil. My fungus, however, would appear distinguished decisively from *B. ranarum* by its abundant production of elongated secondary conidia with adhesive glandular beaks. If similar conidia were formed in *B. ranarum,* they could hardly have been overlooked by the several very competent investigators who have given that species prolonged study. Even if the structure which Eidam interpreted as a "basidium" bearing a small tertiary conidium on its tip, should have been, as Thaxter hinted, a conidium of the secondary type occurring in *Empusa Fresenii,* the pronouncedly tapering shape evident in Eidam's figure, and the absence of any indication of a thick-walled cylindrical part below the globose protuberance, would yet offer features quite alien to my fungus. Further, if the particular "basidium" in question were to be construed as a secondary conidium, its nodding posture would relate it more accurately to the secondary conidia of *E. Fresenii* and *E. lageniformis,* which Thaxter described as "borne obliquely on capillary conidiophores," than to the secondary conidia of the Wisconsin fungus, which like those of *E. Lampyridarum* Thaxt. (20, p. 169–170), among insectivorous forms, are "borne vertically on capillary conidiophores," their axes always being in alignment with the supporting hyphae.

Because its adhesive organs are very unusual among phycomycetes, and, besides, almost certainly imply a destructive biological relationship to some species of animal, the fungus is described as new despite the lack of knowledge concerning its vegetative and overwintering stages.

Basidiobolus haptosporus Drechsler, sp. nov. Conidiis primariis incoloratis, globosis vel late ovoideis, plerumque papilla subtruncata praeditis, vulgo circa 25 μ crassis, in maturitate cum "basidio" inflato violenter emissis, mox hypham fertilem erectam 250–325 μ altam basi 3.5–4.5 μ crassam sursum 2 μ latam saepe porrigentibus et in apice ejusdem conidium secundarium gerentibus. Conidiis secundariis saepe in totum 64–73 μ longis, in cellula viventi et rostro vacuo glutinoso consistentibus; cellula viventi incolorata, elongato-ellipsoidea, recta vel leviter curvata, saepe 50–61 μ longa, 14.5–17.5 μ lata, apice ab rostro septo flavo divisa; rostro tubuliformi, 6–8 μ longo, 2–2.5 μ crasso, muro ejus flavo, magnam partem 0.8–1 μ crasso sed sursum rotundato et valde attenuato et guttula globosa materiae glutinosae flavidae 8–10 μ in diametro vestito. Conidiis ordinum tertii et quarti et quinti modo rarius ad instar primariorum globosis denique cum "basidio" violenter emissis, plerumque ad instar secundariorum elongatis rostratisque sed minoribus, interdum tantam modo 35 μ longis et 8 μ latis, denique rostro eorum interdum tantum 4 μ longo et 1.5 μ lato, et hypha fertili germinationis interdum tantum 150 μ alta, basi tantum 2.2 μ crassa, apice tantum 1.2 μ crassa. Cellula viventi conidiorum elongatorum cujusque ordinis quandoque aliquot septis in plura (interdum 5) loculamenta (sporas) divisa.

Habitat in humo silvestri prope Mercer, Wisconsin.

Primary conidia colorless, globose or broadly egg-shaped, usually provided with a blunt protuberance, commonly about 25 μ in diameter, when fully developed regularly discharged forcibly together with the inflated distal portion of the conidiophore, thereupon often producing a secondary conidium erectly at the tip of an erect conidiophore usually 250–325 μ high, 3.5–4.5 μ wide at the base, and 2 μ wide near the apex. Secondary conidia often 64–73 μ in total length, consisting individually of a living cell and an adhesive apical beak; the living cell colorless, elongated, ellipsoidal, straight or slightly curved, often 50–61 μ long, 14.5–17.5 μ wide, delimited distally from the beak by a thick yellow septum; the apical beak tubular, at maturity devoid of protoplasmic contents, commonly 6–8 μ long and 2–2.5 μ wide, its wall yellow, in large part 0.8–1 μ thick but always very thin at the rounded apex, which is surrounded by a globose mass of yellow adhesive material 8–10 μ in diameter. Conidia of the third, fourth, and fifth orders only seldom resembling the primary conidia with respect to globose shape and violent manner of discharge, being usually similar to secondary conidia in shape and beaked apical modification, though of smaller size, sometimes measuring only 35 μ in total length and 8 μ in width, the beak then often only 4 μ long and 1.5 μ wide, and the erect germ-conidiophore frequently extended by them then sometimes measuring only 150 μ in height, 2.2 μ in basal width, and 1.2 μ in apical width; the living cell in elongated conidia of whatever order occasionally becoming divided by several cross-walls into plural (sometimes 5) cells or sporangiospores.

Occurring in deciduous leaf mold near Mercer, Wisconsin.

UNITED STATES DEPARTMENT OF AGRICULTURE
  BELTSVILLE, MARYLAND

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ANTILUMINESCENT ACTIVITY OF ANTIBACTERIAL SUBSTANCES

FREDERICK KAVANAGH

The usual test for the presence of an antibacterial substance depends upon the inhibition of the growth of bacteria by it, although test methods based upon inhibition of production of nitrite (Goth & Bush 1944), of hemolysin (Rake & Jones 1943), and of reduction of methylene blue (Reid & Brewer 1946) by bacteria have been used. The observation that the luminescence of photobacteria is decreased greatly by certain antibacterial substances but not by others makes the photobacteria useful both for quantitative determinations and for characterization of antibacterial substances.

The antiluminescent test for antibiotic substances was first used by Rake, McKee and Jones (1942) who devised a 30-minute test for aspergillic acid and showed that penicillin was without antiluminescent activity. The test was valuable also for indicating the presence of a small amount of aspergillic acid in the presence of a much larger amount of a penicillin (McKee, Rake & Houck 1944). Nine of fourteen antibiotic substances were shown to be antiluminescent (Rake, Jones & McKee 1943). Only five of the fourteen substances however, were crystalline; and the activities of the others may have been caused in part by impurities. The *Photobacterium* test was used by Atkinson and her colleagues (1944) to follow the progress made in the isolation and crystallization of the antibacterial substance, "penicidin," a product of an unidentified species of *Penicillium*. The speed and specificity of the antiluminescent test suggested that it might have wide applicability in differentiating antibacterial substances as well as for quantitative determinations of some of them.

MATERIALS AND METHODS

Organism. Two strains of *Photobacterium Fischeri* were obtained from G. Rake and used in several preliminary tests. Since the strain labeled "Doudoroff" gave a luminescence considerably greater than the other strain, it was chosen for further use. All data reported in this paper are based on work done with the Doudoroff strain.

Stock Culture. Stock cultures were maintained in an artificial seawater broth in which the bacteria were viable, though not luminescent, for at least six months at 15° C, and on the modified Egorova-Yarmolink (1945) agar (E–Y agar) on which they were luminescent for about one month and viable for more than six months at 15° C.

The E–Y agar, as modified by the substitution of distilled water for the fish extract and Bacto-peptone for the Witte peptone, is: NaCl 30 g., Bacto-peptone 10 g., asparagine 5 g., K_2HPO_4 1 g., $MgSO_4 \cdot 7H_2O$ 0.5 g., agar 20 g., water 1000 ml.; pH after sterilization 6.9. A broth, E–Y broth, was also used in certain experiments; this contained the same concentrations of salts, peptone, and asparagine as the agar medium.

Test Medium. The test medium was the artificial sea-water broth used by Rake, McKee and Jones (1942).

	Artificial sea-water broth
NaCl	26.7 g.
KCl	0.71 g.
$CaCl_2$	1.15 g.
$MgCl_2 \cdot 6H_2O$	5.11 g.
$MgSO_4 \cdot 7H_2O$	6.81 g.
Bacto-peptone	2 g.
Water, distilled	1000 ml.
pH	6.2–6.6

The media were sterilized by heating at 120° C for 15 minutes. A flask containing 100 ml. of medium was inoculated with bacteria taken from an agar slant or with several milliliters of solution from a tube of sea-water broth which had grown for 48 hours or longer at 15° C. The inoculated flask was then incubated at 15° C for 48 hours with frequent shaking to give a brightly luminescent suspension of bacteria.

Temperature of Incubation. Although the curves given by Johnson, Eyring and Williams (1942) showed the luminescence-intensity of *Achromobacter Fischeri* to be five times as great at 25° C as at 15° C, the Doudoroff strain of *P. Fischeri* glows more strongly in both E–Y broth and sea-water broth at 15° than at 25° C. The growth of *P. Fischeri* in artificial sea-water broth, as measured by turbidity of the solution after incubation for 48 hours, was best at 20° C, slightly less at 15° C, about 75 per cent as much at 25° C as at 20° C, and none at 30° C. The luminescence of the cultures grown at 15° C and 20° C was about equal and considerably greater than that of the culture grown at 25° C, although the difference was small after only 24 hours of incubation. Rake, McKee, and Jones incubated their tests at 25° C, and Atkinson and co-workers, using a strain obtained from Rake, at 16° C. The tests to be reported here were incubated at 15° C.

Oxygenation. The full luminescence of a suspension of the bacteria developed only when an adequate amount of oxygen was present. The tubes of a test were shaken to aerate them just before a test was read. Vigorous sidewise shaking of the racks of tubes provided sufficient oxygen.

Concentration of Bacteria. The concentration of the bacteria can influence decidedly the minimum concentration of antibacterial substance that causes suppression of luminescence. This is important only when abso-

lute values are used, as in certain identification procedures. The effect of concentration of bacteria on the sensitivity of the tests will be discussed later when the activities of tolu-p-quinone, aspergillic acid, and patulin are considered in detail.

Serial Dilution Test. Only the geometrical-series dilution test (Rake, McKee & Jones 1942; Kavanagh 1947a) was performed with *P. Fischeri*. In each tube (12 × 75 mm.) was placed 0.5 ml. of the artificial sea-water broth; the serial dilutions of the antibacterial substances were made, and, when all of the dilutions of a set of tests had been made, 0.5 ml. of a 48-hour culture of the *Photobacterium* was added as rapidly as possible to each tube. The racks of tubes were shaken to mix and to oxygenate the solutions and were placed immediately at 15° C. After the tests had been at 15° for the appropriate time, the racks were shaken to aerate the solutions, and the number of non-luminescent tubes was counted. The reading of the test was done with the unaided, dark-adapted eye in an absolutely dark room. The dilution of the antibacterial substance was calculated from the number of non-luminescent tubes, the first tube representing a dilution of 4. Since all the data reported were obtained by the geometrical-series dilution method, the concentrations can be in error by a factor of 2.

Substances and Sources. The antibacterial substances were obtained from the following: aspergillic acid from G. Rake of the Squibb Institute for Medical Research; the antibiotic from *Arctium minus* and citrinin from J. H. Bailey of the Winthrop Chemical Company; 4,6-dimethoxy-toluquinone from Harold Raistrick; dihydrostreptomycin trihydrochloride (M '2216-H2, about 740 μg./mg.) from O. Wintersteiner of the Squibb Institute; gliotoxin and desthiogliotoxin from J. D. Dutcher of the Squibb Institute; "Hogeboom and Craig No. 1" from L. C. Craig; kojic acid from Commercial Solvents Corporation; mycophenolic acid from Harry Sobotka; patulin, penicillic acid and spinulosin from Harold Raistrick; the crystalline salts of the penicillins from the Commercial Solvents Corp.; streptomycin trihydrochloride (M2213, 840 μg./mg.) from O. Wintersteiner; streptomycin trihydrochloride–calcium chloride double salt (109 X 28 C, 715 μg./mg.) and hydrogenated streptomycin trihydrochloride (144 X 390 I, 800 μg./mg.) from the Research Laboratories of Parke, Davis & Co.; and streptothricin (495 units/mg.) from R. T. Major of Merck and Co. The "Hogeboom and Craig No. 1" (Hogeboom and Craig, 1946) was thought by Doering, Dubos, Noyce and Dreyfus (1946) to be identical with their "ustin." The activities of the dihydrostreptomycin and of the hydrogenated streptomycin were identical when tested by the methods used here and are given in the table under dihydrostreptomycin. The 2-methyl-1,4-naphthoquinone and 2-methyl-1,4-naphthohydroquinone diacetate were commercial products and were used

as received. The tyrothricin was a commercial preparation and was a mixture of unknown proportions of gramicidin and tyrocidine. The two naphthoquinones and tolu-p-quinone were Eastman products that had been recrystallized shortly before using. The hydrogen peroxide was prepared by diluting Merck "Superoxol" with water. Biformin (Robbins, Kavanagh & Hervey 1947a), cassic acid (Robbins, Kavanagh & Thayer 1947), and pleurotin (Robbins, Kavanagh & Hervey 1947) were isolated in this laboratory. The biformin was the purest obtained; the others were crystalline.

The activities are expressed as the minimum antiluminescent concentration in micrograms per milliliter and are for the substances as received, except for the streptomycin and dihydrostreptomycin which are expressed as the free base. The solutions were used without sterilization or after passage through a small Seitz filter pad. When the solutions were sterilized, the first 2 ml. through the filter were discarded and the next 3 ml. collected for use. The solutions were used as soon as possible after they were made because dilute solutions of the quinones, pleurotin, and patulin, at least, are not stable indefinitely.

All the solutions, except the penicillins, were stored in the dark at 11° C when not in use. The penicillins were dissolved in pH 6.7 phosphate buffer and stored at 4° C in a refrigerator.

RESULTS AND DISCUSSION

Antiluminescent Activity. The minimum antiluminescent[1] concentrations of the 26 antibacterial substances active against *P. Fischeri* are given for five different periods of incubation in table 1. The observations of Rake, Jones and McKee (1943) obtained at 25° C and after an incubation period of 0.5 hour are included. The antibacterial activities[2] (Kavanagh 1947) of the substances against the Heatley strain of *Staphylococcus aureus* (ATCC 9144) and *Escherichia coli* (ATCC 9637) are given in the last two columns of table 1, for comparison with the antiluminescent activity.

The antibacterial substances can be placed in one of four groups[3] on the basis of the time course of antiluminescent action.

1. Inactive: Arctium minus, 2-methyl-1,4-naphthohydroquinone, the penicillins, kojic acid, mycophenolic acid, and desthiogliotoxin.
2. Inactive at first, or giving incomplete "blacking-out" for 3 hours but

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[1] Procedures for using photobacteria in an antibacterial test are given elsewhere (Kavanagh 1947a).

[2] The 2-methyl-1,4-naphthohydroquinone diacetate was not antiluminescent at 40 μg./ml. and was inactive against *S. aureus* at 800 μg./ml. The desthiogliotoxin was not antiluminescent at 250 μg./ml. and was inactive against *S. aureus* at 500 μg./ml.

[3] The test method does not distinguish between activities of 250 and 256 micrograms/ml. or, for that matter, between 250 and 400 micrograms/ml. The activity listed in table 1 as "> 250" is interpreted as more than 256; how much more is not known.

TABLE 1. *Minimum antiluminescent concentration of antibacterial substances in micrograms per milliliter.*

Antibacterial substance	Photobacterium Fischeri						Staph. aureus	Esch. coli
	10 min.	1 hr.	2 hr.	3 hr.	24 hr.	½ hr.		
"Aretium minus"	>250	>250	>250	>250	>250	15	60	
Aspergillic acid	9	2	2	2	2		8	62
Biformin		0.34	0.34	0.34	0.17		0.3	1.7
Cassie acid	64	64	32	32	16		8	1000
Citrinin	256	128	64	32	16		16	1000
Dihydrostreptomycin	>400	>400	>400	>400	100	......	0.03	0.25
4,6-Dimethoxytoluquinone	16	16	16	16	2		1	250
Gliotoxin	32	32	8	8	2	17	0.12	25
"Hogeboom and Craig No. 1"	>25	25	12.5	12.5	>25		1	>50
Hydrogen peroxide	12	6	6	6	6		8	10
Kojic acid	>2500	>2500	>2500	>2500	>2500		1250	2500
2-Methyl-1,4-Naphthoquinone	3.3	3.3	3.3	3.3	3.3		1.7	200
Mycophenolic acid	>500	>500	>500	>500	>500		250	500
1,2-Naphthoquinone	0.4	0.4	0.8	0.8	1.6		8	25
1,4-Naphthoquinone	0.4	0.4	0.4	0.4	0.4		8	25
Patulin	256	8	2	2	0.5	22	8	8
Penicillic acid	128	64	16	16	4		16	64
Penicillin G	>500	>500	>500	>500	>500		0.016	16
Penicillin X	>>1000	>>1000	>>1000	>>1000	>>1000		0.03	16
Pleurotin	32	16	8	8	16		0.03	
Spinulosin	64	64	125	125	125		1	>500
Streptomycin	>400	>400	>400	>400	100		64	250
Streptothricin	>>300	>>300	>>300	>>300	150	56	0.03	0.25
Sulfanilamide	750	750	375	375	3000	3940	0.1	0.1
Tolu-p-quinone	1	1	1	1	2	0.11	3000	
Tyrothricin	>125			>125	125	>500	1	25
							4	

active after 24 hours of incubation: cassic acid, streptothricin, tyrothricin, streptomycin, and dihydrostreptomycin.

3. Active immediately, the activity increasing with increasing duration of incubation: citrinin, gliotoxin, patulin, and penicillic acid.

4. Activity attaining a near maximum value in one hour or less, and

(a) remains constant or increases slightly in the 3–24 hour period: aspergillic acid, biformin, 4,6-dimethoxytoluquinone, hydrogen peroxide, 2-methyl-1,4-naphthoquinone, and 1,4-naphthoquinone; or,

(b) decreases somewhat on further incubation: 1,2-naphthoquinone, pleurotin, spinulosin, sulfanilamide, and tolu-p-quinone.

Antiluminescent activity seems to be unrelated to a particular type of antibacterial activity. The substances in groups 1 and 2 have neither chemical properties nor antibacterial activities in common. In group 3, the two compounds that were chemically related, patulin and penicillic acid, being unsaturated lactones and unsaturated ketones, showed identical time-courses of antiluminescent action after the first hour. Their relative antiluminescent activities were the same as their relative antibacterial activities against *Esch. coli*. The unsaturated ketone may be the reactive group rather than the unsaturated lactone, since citrinin and the substances from *Arctium minus*, which may be unsaturated lactones, behave in a different manner. In group 4, the quinones, without exception, showed maximum activity within 10 minutes or less after mixing with the suspension of bacteria. The other members of this group did not act quite so rapidly as the quinones. The decrease in activity with increasing time of incubation exhibited by several of the quinones, pleurotin, and sulfanilamide may be due to growth of resistant bacteria, or to destruction of the antiluminescent substance.

Antiluminescent activity could not be attributed to a particular grouping of atoms since these antiluminescent substances did not have a group common to all. The molecules could not be modified much, however, without destroying activity, as was indicated by the removal of the sulfur atoms from gliotoxin and the reduction of 2-methyl-1,4-naphthoquinone to the naphthohydroquinone. These changes also removed the antibacterial activity against *Staph. aureus*. Antibacterial activity, while it increases the probability of antiluminescent activity, does not make it certain, for the penicillins and the substance from *Arctium minus* were not antiluminescent. Most of the substances listed in table 1 presumably react with sulfhydryl groups since their antibacterial activity is destroyed by cysteine. However, sulfhydryl reactivity alone is not sufficient to cause antiluminescent activity, for both penicillin and the agent from *Arctium minus* react with cysteine and both are inactive as antiluminescent agents. Both streptomycin (which is inactivated by sulfhydryl) and dihydrostreptomycin (which is not inactivated by sulfhydryl) are feebly antiluminescent.

Although the penicillins were inactive in this antiluminescent test, they

showed good activity (8–16 μg./ml.) in an antibacterial test using *P. Fischeri* (Kavanagh 1947a). Thus the penicillins were active against growing cultures of *P. Fischeri* and inactive against cells that were not growing just as Lee, Foley and Epstein (1944) showed was true for *Staph. aureus*.

Quinones. Because of the high and immediate activity of tolu-p-quinone, it was of interest to compare substituted and unsubstituted quinones as inhibitors of luminescence. Tolu-p-quinone and two derivatives, and two naphthoquinones and one derivative, were tested at equal molecular concentrations. The minimum inhibitory concentrations in micromoles per liter after 10 minutes and 1 hour were 2.5 for tolu-p-quinone, 1,2-naphthoquinone, and 1,4-naphthoquinone; 20 for 2 methyl-1,4-naphthoquinone; 100 for 4,6-dimethoxytoluquinone, and 330 for 3,6-dihydroxy-4 methoxytoluquinone (spinulosin). Substitution decreased the antiluminescent activity of the quinones but not the speed with which they reacted. The number and kinds of substitution are too few to justify extensive speculation about the reasons for the differences in activity between substituted and unsubstituted quinones.

Antibacterial Action of Antiluminescent Substances. Possibly the large increase in the activity of aspergillic acid, biformin, and patulin when the incubation period exceeded 10 minutes occurred because the bacteria were killed. To test this assumption, 0.007 ml. of broth was transferred from each "blacked-out" tube to 7 ml. of sea-water broth or E–Y broth and the tubes of broth incubated at 15° C for four days with frequent inspections for signs of growth of bacteria. The length of the incubation period before 'the first appearance of luminescence in the transfers was approximately proportional to the number of living cells in the "blacked-out" tube from which the transfer was made. A concentration of antibacterial substance that killed 99 per cent of the bacteria was considered to be bactericidal. The bactericidal concentration determined by this method may have a large error and the concentrations found in different experiments differed considerably. A representative sample of the results is given in table 2.

TABLE 2. *Bactericidal and Antiluminescent Activities of Four Antibacterial Substances.* Concentrations are expressed in micrograms per milliliter.

Substance	Bactericidal				Antiluminescent			
	10 min.	1 hr.	3 hr.	24 hr.	10 min.	1 hr.	3 hr.	24 hr.
Aspergillic acid ...	..	> 250	> 250	60	250	16	8	8
Biformin	...	> 3.4	> 3.4	3.4	> 3.4	0.4	0.2	0.2
Patulin	.	64	64	8	250	8	2	0.5
Tolu-p-quinone	4	4	4	8	0.5	1	1	2

The bactericidal concentration of three of the substances is considerably higher than the antiluminescent concentration. The slow development of antiluminescent action of aspergillic acid, biformin and patulin, then does not result from death of the cells. Tolu-p-quinone is unique in causing death so quickly and at such small concentration which, however, is about 8 times the 10-minute antiluminescent concentration. The lethal concentration of each substance decreased with increased duration of exposure to it.

Concentration of Bacteria. The effect of concentration of bacteria upon activity of aspergillic acid, patulin, tolu-p-quinone, 4,6-dimethoxy-toluquinone, and spinulosin was determined for undiluted cultures and for cultures diluted[4] 8 times with sea-water broth. The diluted culture required the same concentration of 4,6-dimethoxy-toluquinone; from one-half to one-

TABLE 3. *Antiluminescent concentration of tolu-p-quinone in micrograms per milliliter for four different concentrations of bacteria.*

Relative con-centration of bacteria	Duration of Incubation of					
	P. Fischeri					Staph. aureus
	10 min.	1 hr.	2 hr.	3 hr.	24 hr.	24 hr.
1	0.5	0.5	0.5	0.5	1	4
0.5	0.25	0.25	0.25	0.25	1	4
0.25	0.125	0.125	0.25	0.25	0.5	2
0.125	0.0625	0.0625	0.0625	0.0625	0.5	4

fourth the concentration of aspergillic acid, patulin, and spinulosin; but only one-eighth the concentration of tolu-p-quinone required by the undiluted culture. The exact proportionality between concentration of bacteria and antiluminescent concentration of tolu-p-quinone was investigated further by measuring the inhibitory concentration for four concentrations of bacteria with the results given in table 3.

For both the ten-minute and one-hour inhibitions, the number of molecules of tolu-p-quinone per bacterial cell required to inhibit luminescence was constant at about 10^7. The data presented in table 3 certainly support, as well as can be expected of the method, the hypothesis that it is the number of molecules per bacterial cell and not the concentration of tolu-p-quinone that determines inhibitions of luminescence but not the antibacterial action against *Staph. aureus*. To prove this, more exact methods of measuring inhibition than were used in this work must be used.

These results emphasize the necessity of using the same concentration of bacteria in all determinations if the absolute value of the inhibitory concen-

4 The luminescence of the diluted culture was very faint and the tests were difficult to read with consequent increased uncertainty of the end-point.

tration is to have meaning. An alternate and preferable procedure, to be used when possible, is to include standards in each series of assays and to compute the concentration of the unknowns in terms of the standards. It is also quite obvious that the standard must be the substance that is being assayed.

Mode of Action. The luminescent mechanism of bacteria has intrigued a number of investigators, some of whom have developed rather elaborate and detailed theories about it. Studies of the quenching of luminescence by a diverse group of chemical substances and by the effects of temperature and pressure on the degree of inhibition have supplied the data needed to test several hypotheses of inhibition of luminescence (Johnson et al. 1945). The antibacterial substances furnish a chemically different, and much more active, group of inhibitors of luminescence than those that have been used. Aspergillic acid, for example, is about 1000 times as effective aь an equal molar concentration of sulfanilamide in inhibiting the luminescence of *P. Fischeri.*

Consideration of three of the types of inhibition of luminescence shown by the antibacterial substances suggests that the inhibition can occur at three, if not more, different points in the chain of reactions connecting the primary energy-furnishing metabolite with the emission of light.

The inhibition of luminescence by tolu-p-quinone is so rapid that it is complete by the time the solution of tolu-p-quinone and the suspension of bacteria are completely mixed. If the tolu-p-quinone competed with luciferin for space on the luciferase molecule, the effect of the addition of the quinone ·could be immediate. In competition between quinone and luciferin, the quinone, at a sufficiently large concentration, could occupy nearly all of the space on the luciferase thus permitting only inappreciable luminescence. The minimum concentration of tolu-p-quinone that causes complete blacking-out, as judged by visual observation, is 10^{-8} mole per milliliter which is, however, about 10^7 molecules per bacterium.

Luciferin is known to be destroyed by oxidizing agents (Anderson 1936). Toluquinone and the other quinones are oxidizing agents and could be anti-luminescent by oxidizing the luciferin. The exact proportionality between the number of bacteria (and presumably of the number of molecules of luciferin) and the number of molecules of tolu-p-quinone that stops luminescence would be expected if the luciferin were oxidized by the toluquinone. However, if the normal reduction potential of luciferin given by Harvey (1941) is correct, the 1,4-naphthoquinone would not be nearly as effective in oxidizing luciferin as tolu-p-quinone and yet these two quinones are equally antiluminescent. When all six of the quinones are considered, there is no correlation between the normal reduction potential and minimum antiluminescent concentration just as there is no relation between the poten-

tial and the antibacterial activity against *Staph. aureus* and *Esch. coli* (Page & Robinson 1943; cf. table 1).

The inhibition of luminescence caused by aspergillic acid develops much slowly than that caused by tolu-p-qpinone. The time required, from one-half to one hour, suggests that the chain of reactions was blocked by the aspergillic acid at a point for enough removed from the final stages of the luminescent reaction to permit luminescence for one-half hour before the accumulated store of intermediates was exhausted. Once the critical concentration of aspergillic acid has been reached, increasing the concentration does not lessen the time required for the development of the inhibition of luminescence. The minimum inhibitory concentration of aspergillic acid is $2 \cdot 10^{-8}$ moles per milliliter, or about the same as that of tolu-p-quinone.

The action of citrinin, gliotoxin, patulin, and penicillic acid seems to be different from that of the other two groups of substances discussed. An immediate inhibition by high concentrations of the antiluminescent substance, presumably, is the result of blocking of a step near the final one leading to emission of light. The inhibition developed slowly at low concentrations could result from blocking a step far removed from the final one. Since the effect of this blocking develops slowly and the inhibitory concentration decreases with increasing time of exposure to the inhibitor, the inhibition could be one that decreased the rate of, but did not stop, the production of an essential intermediate. The higher the concentration of inhibitor, the greater the decrease in rate of production of intermediate and the sooner the concentration of it is reduced by normal metabolic processes to the point where it becomes the factor limiting luminescence.

Characterization of Antibacterial Substances. One of the problems that confronts anyone who works with the antibacterial substances formed by many different organisms is identification of each of the substances. They need to be characterized sufficiently well by methods applicable to impure preparations that their identity, or lack of it, with known substances can be established. Antiluminescent activity is particularly useful because it frequently is not proportional to antibacterial activity of the substance as measured against the usual Gram-positive and Gram-negative bacteria. In applying the test to culture fluids and other preparations containing unknown concentrations of the active substance, relative activities are computed and compared with those computed from the data in table 1. Two kinds of relative activities are useful, those relative to the 24-hour antiluminescent activity, and those relative to antibacterial activity against Gram-positive or Gram-negative bacteria. For example, the concentration that is antiluminescent at 24 hours can be compared with the concentration that inhibits growth of *Staph. aureus* for 24 hours.

SUMMARY

1. Twenty of the 27 antibacterial substances were antiluminescent when tested against *Photobacterium Fischeri*. They varied greatly in speed of action and in effectiveness.

2. The substances that were antiluminescent did not have a common chemical group to which the activity could be ascribed. Ability to react with sulfhydryl groups was not a sufficient condition for antiluminescent activity; the quinones inhibited luminescence most rapidly; the three unsubstituted quinones were equally active, and were active at a low concentration.

3. The antiluminescent activity of a compound does not necessarily parallel its antibacterial activity against *Staphylococcus aureus* or *Escherichia coli* but frequently does.

4. Several of the antiluminescent substances are bactericidal.

5. The minimum antiluminescent concentration of tolu-p-quinone was found to be exactly proportional to the number of bacteria present. The activity of other substances was much less affected by bacterial concentration.

6. The antibacterial substances seem to affect the luminescent mechanism at two or three places in the chain of events that results in luminescence.

7. Antiluminescent activity may be useful in characterizing an antibacterial substance before it has been isolated in pure form and in following it quantitatively during isolation and purification.

NOTE: After the above paper had been sent to the printer, the following material was tested.

A sample of pure, crystalline helvolic acid (fumigacin) was obtained from E. A. Doisy of St. Louis University. The test was not satisfactory because the test-medium was saturated with helvolic acid even at a concentration of 5 µg/ml. The fumigacin which was active at 273 µg/ml. (table 1) was contaminated with about 20 per cent of gliotoxin fractions (Menzel, Wintersteiner & Hoogerheide 1944; Waksman & Geiger 1944). The presence of 6.2 per cent of gliotoxin in the sample could account for the activity.

The following line should be inserted in table 1 between gliotoxin and ''Hogeboom and Craig No. 1.''

| Helvolic acid | >5 | >5 | >5 | >5 | >5 | 273 | 1 | >1000 |

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TORREYA

BOOK REVIEW

A Textbook of Systematic Botany. (3rd ed.) By Dean B. Swingle. i–xv, 1–343. *f. 1–106*. New York: McGraw-Hill Book Co., Inc. 1946. $3.50.

There is perhaps little point in completely reviewing this edition of a well known and widely used textbook except to examine it in the light of the publisher's note on the dust jacket which states that it "meets the demand for a textbook that approaches the subject by way of genetics, ecology, cytology, and geography."

While it is admitted that mention of these items as they affect a study of systematics does differentiate this edition from the earlier ones, one cannot help but regret that even more consideration was not given them. However, it should perhaps be held in mind that it may not have been Professor Swingle's intention that this text be addressed to advanced students but, rather, to beginners who, presumably, would have no more background than a first course in General Botany. This, therefore, would limit the extent to which he could pursue these highly important topics. The mere fact that one finds them more than casually mentioned in a textbook of systematics is encouraging.

In passing, it would seem that attention should be called to a feature by no means limited to the present text. In my own way of thinking it is a too-common practice to attempt to combine a course in Systematics with one in Local Flora. In fact, in all too many institutions the course listed in the catalog as "Plant Taxonomy" usually simmers down to one in Local Flora—the identification of the plants of the immediate area. A familiarity with the plants of one's region is a needed part of any taxonomist's training, but it certainly is not plant taxonomy in its broadest sense.

It is immediately obvious, when an author approaches the problem of a general textbook in taxonomy, that there must be some selection of subject matter. But, if the teacher choosing this particular text sticks closely to it, one wonders just what concept the student would have óf angiospermous groups and their relationships. As examples (and these are taken quite at random): The Cruciferae are treated, but nowhere is there any mention of the basic complex from which they seem to have been derived—the extensive, mostly tropical, and often arborescent Capparidaceae. The Aceraceae occupy several pages of text and a full plate of figures; but nowhere could I find mention of the much larger and more important Sapindaceae, their tropical precourser, except under the Fagaceae. The Asclepiadaceae apparently required two pages of figures; but the name Apocynaceae does not even appear as a word in the Index. In the Violaceae the genus *Viola* is taken as representative, together with the note that "a few tropical forms are shrubby"—with all omission of the fact that the basic, tropical members of the family are forest trees. We would fully agree that the Berberidaceae with their 150 species are worthy of inclusion; but one somehow misses certain families, such as the Myrtaceae with their more than 4,000 species, many of them of considerable economic importance, with others com-

monly grown as ornamentals in various parts of the United States. Obviously, not all the families of spermatophytes could be included in such a text, but it would seem that at least the larger and more important (even if mainly tropical) ones merit at least brief mention.

The foregoing is not so much intended as a criticism of this text as of all those which attempt a "general review" of the angiosperms on the basis of local, north temperate examples. By taking this viewpoint the beginning student in taxonomy unknowingly falls into a fundamental error—that of all too easily assuming that the plants which he finds on his brief field trips are representative of the families studied—or, for that matter, of the world's flora. This viewpoint, so far as leading to an understanding of the origin and development of the north temperate floras is concerned, is much akin to looking through the wrong end of a telescope.

This almost purely local viewpoint may also bring about another result. With so limited a concept of the background and ancestry of temperate groups—and finding these apparently adequately treated in the local manuals—the beginning student is prone to get the notion that there is little that remains to be done in taxonomy and so drifts into other fields. Actually, our taxonomic studies have scarcely more than scratched the surface of the majority of the great groups of plants. It would seem that this might well be one of the items which should be stressed in any textbook of systematic botany.—W. H. CAMP.

PROCEEDINGS OF THE CLUB

Minutes of the Meeting of April 16, 1947. The regular afternoon meeting of the Torrey Botanical Club was called to order by Dr. Jennie L. S. Simpson at 3:35 p.m. in the Members' Room of the New York Botanical Garden. Sixteen members and friends were present. The minutes of the previous meeting were approved as read. No business was transacted and Dr. Simpson introduced Dr. A. B. Stout who spoke on "Data on the Heredity of One-way Hybridization." Following a discussion period the meeting was adjourned at 4:45 p.m. Refreshments were served by members of the Garden staff.

Respectfully submitted,
LIBERO AJELLO,
Recording Secretary

Minutes of the Meeting of May 14, 1947. The regular evening meeting of the Torrey Botanical Club was called to order by the President Dr. George H. Shull, at 8:30 p.m. at Hunter College. Thirty members and friends were present. The minutes of the previous meeting were approved as read. Four Associate Members and six Annual Members were elected.

No further business was transacted and Dr. Shull introduced Dr. Michael Evenari who spoke on "The Physiological-ecological Behavior of Desert Plants." This stimulating and highly informative talk was followed by a discussion period. The meeting adjourned at 9:30 p.m.

Respectfully submitted,
LIBERO AJELLO,
Recording Secretary

Special Meeting of May 27, 1947. On this date the Torrey Botanical Club gathered in the Faculty Dining Room of Hunter College for a dinner meeting in honor of the retirement of three active members: Dr. B. O. Dodge, Dr. Arthur H. Graves, and Dr. Alfred Gundersen. The President, Dr. George H. Shull, presided and about 60 members and guests were seated.

After the dinner Dr. Shull called on Dr. W. H. Camp to introduce Dr. Gundersen. Dr. Gundersen told about his early interest in several sciences, and especially his interest in botany, while still living in Norway. After coming to this country his interest in plants became more pronounced and particularly his interest in classification. Several allusions were made to his new book which is in press. He urged the younger botanists, and especially teachers, to continue field work as one of the essential methods of really learning to know plants.

Next Dr. Shull called on Dr. John A. Small to introduce Dr. Graves. Dr. Graves made some additional complimentary remarks about Dr. Gundersen and his book. He also told of his own early interest in botany, and of his work in teaching the trees and shrubs course at the Brooklyn Botanic Garden. He was about to speak of his research, but noticing that his time was up he concluded his remarks immediately.

Finally Dr. H. A. Gleason was asked to introduce Dr. Dodge. Dr. Dodge told of his early connection, and that of Mrs. Dodge, with the Torrey Botanical Club. While he was a graduate student at Columbia University, they handled between them the work of the Secretary, Treasurer, and Bibliographer of the Club. After a sojourn in Washington, D. C., Dr. Dodge said he returned to the New York Botanical Garden on an agreement with Dr. Britton that he look after the plant diseases of the Garden, and the rest of the time he could devote to his own research. From that research has developed the genetics of *Neurospora.*

After the more formal part of the meeting, these three retiring members received congratulations from their many friends as the meeting broke up.

H.H.C.

FIELD TRIP REPORTS

JUNE 30, 1946. LAKEHURST, N. J. ''It would have been impossible to have had a more perfect June day in the Pine Barrens. Temperature 55°, a brisk west wind, and no flies! The Bald Cypress was shown to the members at the start of the trip. The origin of the tree is in doubt. No rarities were found, but a number of the characteristic species of the Pine Barrens were located.'' A list was compiled of 32 plants seen in flower at this date. Attendance 3. Leader, David Fables.

AUGUST 25, 1946. BEACH HAVEN, N. J. A list of over 20 birds was compiled from observations on the mud flats at the southern tip of Long Beach Island and nearby Tuckerton Meadows. Sea Lavendar and Pink Sabattia were at the height of their bloom. Attendance 15. Leaders, David Fables and Charles Rogers.

NOVEMBER 3, 1946. POINT PLEASANT, N. J. ''A heavy rain poured down all day but failed to dampen the enthusiasm of the members present. All were amply repaid for their efforts when 50 Gannets gave a very nice exhibition of diving for fish from heights of 10 to 50 feet above the surface of the ocean. An immature Jaeger was seen chasing a gull. The species seldom comes as close to shore as it did that day. 30 species of birds were observed.'' Attendance 15. Leader, David Fables.

MARCH 23, 1947. POINT PLEASANT, N. J. Although our Field Schedule was delayed in distribution until after this date, a group made the trip anyway and reported a successful study of waterfowl of the season. Plant life at the shore was still dormant but those plants usually observed in that aspect were found. Attendance 7. Leader, David Fables.

APRIL 13, 1947. NEW YORK BOTANICAL GARDEN. With the conservatories supplying the plant materials, a study of ''Flowers of a Florida Garden and Their Stories'' was offered. Our thanks to the Botanical Garden and their personnel for assistance. Attendance 8. Leader, Mrs. George Anderson.

APRIL 20, PLAINFIELD, N. J. A walk of 8 or 9 miles including valleys and ridges of the Watchung Mtns. A late spring is forecast by the small numbers of expected plants actually found in flower. Nevertheless, a good representation of the spring flora was seen

and a considerable variety of birds were recognized. Attendance 4. Leader, Dr. H. B. Gordon.

APRIL 20. NEW BRUNSWICK, N. J. A morning walk along the south bank of the Raritan River for collecting was followed by lunch and an afternoon of studying the material in the botanical laboratory of New Jersey College for Women. The following species of liverworts were found to have been collected: *Calypogeia trichomanis* (L.) Corda, *Cephalozia bicuspidata* (L.) Dum., *C. connivens* (Dicks.) Lindb., *Conocephalum conicum* (L.) Wiggers., *Gymnocolea inflata* (Huds.) Dum., *Odontoschisma prostratum* (Sw.) Trev., *Pellia epiphylla* (L.) Corda, and *Reboulia hemisphaerica* (L.) G. L. & N. Attendance 14. Leader, William Rissanen.

APRIL 27. PRINCETON, N. J. A walk along Stony Brook and through the associated woods. An extended list of early spring plants was compiled, the majority of the plants being in flower. Birds received the attention of several members of the group. An impromptu tour of the Princeton Campus was enjoyed by those willing to tramp the additional mileage. Attendance 20. Lealers, Peg and Charles Rogers.

MAY 4. WEST PARK, N. Y. "In spite of the terrible weather, we had 22 at the Slabsides trip today! We built a fire in John Burroughs' fireplace and dried off and listened to Julian Burroughs read some of his father's poems and tell stories of his childhood, as well as philosophize on Nature, on hawks that fly through his garage windows while the windows are closed, and on life in general. 'A good time was had by all,' was the general consensus.'' Leaders, Farida A. Wiley and Harold N. Moldenke.

MAY 4. ALLAIRE, N. J. The Dutchman's breeches were there but nearly inundated, as were the five participants. Leader, Mr. V. L. Frazee.

MAY 4. WATCHUNG RESERVATION, UNION COUNTY, N. J. Two early bird walks; five late morning walks for ecology, trees, minerals, mosses, and other plants; a fine display in the museum; and a talk by the Naturalist from Trailside Museum at Bear Mtn. opened the nature study program and the museum in the usually impressive manner. Attendance was about 30 water resistant nature lovers plus uncounted visitors after the sun came out. Leader, Mildred Rulison and assistants.

MAY 10–11. PALISADES INTERSTATE PARK, N. Y. A pleasant weekend with congenial folks at Camp Thendara. Bird study and spring flowers were enjoyed by invitation of the New York Section of the Green Mountain Club. Attendance 28. Leader, Mrs. Laura W. Abbott.

MAY 11. GREENBROOK SANCTUARY OF THE PALISADES. There was a good response to the appeal for aid in the biological survey of this sanctuary. A general survey was made and a start on listing the species in the various groups. The further participation of local botanists is urged. Attendance 19. Leaders, Mrs. F. G. Dunham and G. G. Nearing.

MAY 16–18. BRANCHVILLE NATURE CONFERENCE, CULVERS LAKE, N. J. The full program of activities announced in the "Field Schedule" was carried out. Reports made at the closing meal indicated that a good deal had been accomplished in the field and indoors. Our hearty thanks to everyone. There were only about half the usual number present and a program geared for 100 does not work out satisfactorily with little more than half that number. The question of whether this Conference has now served its usefulness and should be discontinued is now under consideration. Anyone having ideas on the subject should report to the Chairman of the Field Committee. Attendance 61. Leaders, Messrs. James Hawley, Wallace Husk, and instructors announced.

MAY 17. CHEESEQUAKE STATE PARK, N. J. This trip initiated a collection of the hepatics to be found in the Park. Eight species were recorded and will be included in the more comprehensive report on the area. In addition, eight new birds were added to the list for Cheesequake. Attendance 5. Leaders, Louis Hand and William Rissanen.

MAY 17. CEDAR BROOK PARK, N. J. Our leader, who is Horticultural Chairman of the Plainfield Garden Club, has been remarkably successful in working up the cooperation of

other clubs and of the Union County Park Commission to the point where several plantings have been made in this park. They have all had competent supervision and really good care, considering the conditions of recent years. We were embarrassed about the attendance but our appreciation was the greater. Attendance 3. Leader Miss H. R. Halloway.

MAY 25. AUDUBON NATURE CENTER AND FAIRCHILD WILD FLOWER GARDEN, GREENWICH, CONN. Those participating made good use of their check lists for birds and saw many plants of interest. It was nice to meet the new director, Charles Mohr, already known to some of us by his fine work at the Academy of Natural Sciences of Philadelphia.   Attendance 15. Leader, Mrs. Henrietta Dotson.

JUNE 7. ATLANTIC BEACH, LONG ISLAND. A joint outing with the Department of Natural History of Brooklyn Institute. A killdeer's nest and live oysters were seen. Sea anemones and some species of land snails were recorded for the first time here. A beginning of the mud flat succession was observed, a miniature salt marsh having appeared on a low area previously held exclusively by *Salicornia*. Attendance 14. Leader, Grace Petersen.

JUNE 9–12. SOUTHEASTERN KENTUCKY. Thirty people assembled at the Mountain Preachers' School at Clear Creek Springs on Monday, June 9. Dr. Lucy Braun gave an illustrated lecture that evening setting the plan of the three days to follow. Tuesday was spent seeing the Mixed Mesophytic Forest at two elevations on Log Mtn. in the morning. After lunch the Hemlock Garden and two other stands on Pine Mtn. were visited, the last at a fire tower, afforded an excellent opportunity to see the geological setting in which the various plant communities had developed. The stands of virgin timber of many species, great height, and enormous basal size were most impressive. Tulip tree, magnolias, and flame azalea added the beauty of their flowers, as did many of the ground plants.

Wednesday provided a 200-mile drive with carefully documented itinerary so that all points of interest to the group could be seen without driving in close formation. This route led up the Cumberland valley, over Pine Mtn., through the hill country of the Cumberland Plateau, to the cliff region of the Red River at Natural Bridge, Ky., where Hemlock Lodge provided us excellent accommodations. Thursday was devoted to the forests of the cliff region and Red River valley. These ranged from pine forests reminiscent of New Jersey (We even saw *Pogonia divaricata*, as rare in Kentucky as in N. J.) to various well developed aspects of the Mixed Mesophytic Forest. The last stop was a fitting climax for around an undercut cliff formation known as a ''stone house'' we saw filmy fern and a co-type locality for *Solidago albapilosa* Braun. Leader, Professor Lucy E. Braun, University of Cincinnati.

Local Flora Committee. At the May meeting of the Council, the Chairman of the Field Committee was also made Chairman of the Local Flora Committee of the Club. It is planned to continue the joint chairmanship at least pending a decision as to the province of each committee and the possible desirability of combining the two. The Field Committee has probably overlapped the Local Flora Committee somewhat in its activities. Members who wish to suggest projects for either committee, or who desire changes in their activities are urged to communicate with the Chairman, John A. Small, N. J. College for Women, New Brunswick, N. J.

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A COMPARATIVE CYTOLOGICAL STUDY OF THREE SPECIES OF THE CHENOPODIACEAE[1]

SISTER MARIE BERNARD WITTE, O.S.F.[2,3]

INTRODUCTION

While studying root tips of *Spinacia oleracea*, Stomps (1910) observed many cells having 2n, 4n, 8n, and rarely 16n chromosomes. The term polysomaty was given to this phenomenon by Langlet (1927). Polysomaty may be defined as that condition wherein one or more tissues of a diploid organism contain some cells of varying degrees of polyploidy. The polyploid condition arises by double reproduction of the chromosomes and at least one mitotic division always intervenes between two successive double reproductions. The process is indeterminate and continues as long as growth and differentiation occur. The multiple complex cells in *Culex pipiens* described by Berger (1938) can not be considered polysomaty because no mitotic divisions occur between the repeated double reduplications. Polyploidy as found to occur in the shoot and leaf of *Allium cepa* (Berger & Witkus 1946) and in the root tips of *Mimosa pudica* (Witkus & Berger 1947) likewise can not be considered polysomaty because in these cases the condition is determinate and exists for only a definite short period in early development.

Polysomaty has been found in a number of plants many of which belong to the family *Chenopodiaceae*. *Spinacia oleracea*, a member of this family, has been studied several times in order to determine the origin of the polysomatic condition. Gentcheff and Gustafsson (1939) and Berger (1941) have given the most satisfactory and complete explanation of its origin.

As a result of these more recent observations it was suggested that a reinvestigation be made of several species of the *Chenopodiaceae* to determine if polyploid cells originated in the same manner in these plants as in *Spinacia oleracea*. Other cytological phenomena were observed during this reinvestigation and an attempt was made to correlate the histological and developmental structures of the root with the cytological conditions.

There is some confusion in the nomenclature of species, even of some genera, in this family of plants. Two of the species included in this study are listed differently by taxonomists. Some consider *Atriplex patula* and *Atriplex hastata* as two separate species. Others claim that *Atriplex hastata*

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is only a variety of *Atriplex patula*. It is this latter classification which the writer has adopted. Again some taxonomists consider *Kochia trichophylla* as a variety of *Kochia scoparia*, but the majority consider these as two separate species. This confusion in taxonomy may be responsible for the lack of agreement in the chromosome numbers reported for some species of this family. Different numbers have been reported for *Atriplex patula* and *Chrenopodium album*, two species studied in the present work.

<center>MATERIALS AND METHODS</center>

Atriplex patula, Chenopodium album, and *Kochia trichophylla* were used in this study. Plants of *Atriplex patula* were collected at Pelham Bay, New York, in late September and transplanted to the greenhouse. Some seeds were removed and dried; others were allowed to fall upon the soil and give rise to new plants the following February and March. Plants of *Chenopodium album* were gathered on the University campus and were likewise transplanted to the greenhouse. Seeds of *Kochia trichophylla* were purchased from Stumpp and Walter Company, New York.

The identifications of *Atriplex patula* and *Chenopodium album* were verified by Dr. H. A. Gleason of the New York Botanical Gardens, whose helpful assistance is most gratefully appreciated.

Primary root tips were obtained by germinating seeds on moist filter paper or moist sand. Secondary root tips were obtained either by digging up plants directly from the soil and cutting uninjured roots or by placing the plants in tap water for a few days and then removing the tips.

Root tips for smears were fixed in the ordinary alcohol–acetic acid fixative and stained with aceto-orcein. Those for sections were placed in Craf fixative and stained in iron-alum haematoxylin. Some sections used for the study of root structure were stained by Foster's tannic acid and iron chloride method. Sections were cut 8 and 10 μ thick.

<center>OBSERVATIONS</center>

Root Structure.   The roots of *Atriplex patula, Chenopodium album,* and *Kochia trichophylla* are in general very similar in structure. The roots of *Atriplex patula* were studied most thoroughly and the description given here applies principally to this plant. The other two plants will be described only in so far as they differ from *Atriplex patula*.

Differentiation of the three primary regions, plerome, periblem, and dermatogen, begins approximately 120 μ from the tip and the three layers are completely established at 150 μ. About 180 μ from the tip the outer periblem cells enlarge and this enlargement extends posteriorly for approximately 400 μ to the region where differentiation occurs (figs. 1, 2, 3, 4).

The innermost region of the meristem, the plerome, is composed of a few

large cells surrounded by small tightly packed cells. Few intercellular spaces were observed in this region of the root.

In the periblem there are from three to seven rows of cells. Beginning near the root tip with correspondingly few rows, the number of rows increases gradually until a maximum number is reached at about 400 μ from the tip. The number of rows of periblem cells is higher in secondary roots than in primary roots while the size of individual cells is greater in primary than in secondary roots. The larger periblem cells are always more distal from the center of the root than smaller cells and are frequently separated from each other by large intercellular spaces. The two inner rows of periblem cells are conspicuous by the fact that their cell walls are often arranged in such a position as to give the appearance of right angles where four cells are in contact. This condition was not so regular in *Chenopodium album* or *Kochia trichophylla* as in *Atriplex patula*.

The dermatogen, which consists of a single layer, has both large and small cells. In this region also the cells of primary roots are larger than those of secondary roots.

In all three plants the meristematic region is quite short. Inner periblem cells could always be found undergoing division farther back in the root tip than middle and outer periblem cells. The meristematic region in *Atriplex patula* is slightly longer than the same region in *Chenopodium album* and *Kochia trichophylla*. In *Atriplex patula* most divisions cease between 500 and 600 μ while in *Chenopodium album* and *Kochia trichophylla* very few divisions were observed beyond 500 μ.

Polysomaty. Different authors had reported 18 and 36 as the diploid number of chromosomes in *Atriplex patula* and 18, 36, and 54 as the diploid number in *Chenopodium album*. The present study showed clearly that 18 is the diploid number in *Atriplex patula* and 36 the diploid number in *Chenopodium album*. A chromosome count of eighteen for *Kochia trichophylla* agreed with all previous reports (figs. 5, 6, 7).

The inner layer of periblem cells in all instances contained only diploid cells. Mitotic divisions were first encountered at about 80 to 90 μ from the tip. Cells at the apex of the meristematic region were more or less uniform in size and were diploid. About 250 μ from the tip the periblem cells began to enlarge in the outer layers and from that region on tetraploid cells with paired chromosomes were observed in all three species. In *Atriplex patula* some tetraploids with unpaired chromosomes as well as a few octoploid cells were observed, but no octoploids were seen in *Chenopodium album* or *Kochia trichophylla*. The octoploids always had paired chromosomes and occurred in the next-to-the-outermost row of periblem cells (fig. 8). In the regions where octoploid cells were observed, all cells of the outer layers of the peri-

blem were large. Many of these cells were not in division, but were probably polyploid, tetraploids and octoploids. The cells in this region were greatly vacuolated (fig. 9). Tetraploid cells were also found in the dermatogen layer in *Atriplex patula* and *Chenopodium album* but not in *Kochia trichophylla*.

At prophase in all three species diploid cells showed long single chromosomes scattered throughout the nucleus. Paired tetraploid and octoploid cells showed, at the earliest visible prophase, long relationally coiled pairs of chromosomes (figs. 10, 11, 12, 13). These paired chromosomes were separated throughout their entire length. In *Chenopodium album* few cells underwent a double reduplication. In this species cells which gave rise to the polyploid condition had a prolonged prophase stage and only a few polyploid metaphases were found.

Figure 14 shows a large cell from *Atriplex patula* with the metaphase chromosomes closely paired. In a few instances (fig. 15) the paired chromosomes still showed their close association at early anaphase. No active pairing of chromosomes at late anaphase or early telophase was observed.

Since polysomaty had previously been reported for *Kochia trichophylla* the present study on this species was concerned with the origin of polyploidy and a comparison of the details of the process with that found in *Atriplex patula, Chenopodium album*, and *Spinacia oleracea*. In *Kochia trichophylla* there were considerably more cells in the early paired prophase stage than had been observed in *Chenopodium album*. The early paired prophase condition was also much clearer in *Kochia trichophylla* than in *Chenopodium album*.

In all three species polysomatic divisions were found in old as well as in young roots, both primary and secondary.

Early Separation of Chromatids in Kochia trichophylla. Frequently at late prophase the chromatids in *Kochia trichophylla* were some distance apart (fig. 16). That this precocious separation of chromatids was not due to excessive pressure in smearing was shown by the fact that it was likewise visible in sectioned material.

Prior to reaching the metaphase plate the chromatids were completely separated so that at metaphase each chromatid could be seen lying close to, but separated from, its sister chromatid. Figure 16 shows the spindle-attachment regions clearly separated. Other cases were observed in which the

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Explanation of figures 1-7

FIGS. 1-4, photomicrographs of cross sections of root tips of *Atriplex patula* taken respectively at 180, 240, 300, and 360 µ from the tip. Magnification × 200. FIGS. 5-7, × 1500. FIG. 5, late prophase diploid cell of *Atriplex patula,* aceto-orcein smear preparation. FIG. 6, late prophase diploid cell of *Chenopodium album,* iron-alum haematoxylin section. FIG. 7, late prophase diploid cell of *Kochia trichophylla,* aceto-orcein smear preparation.
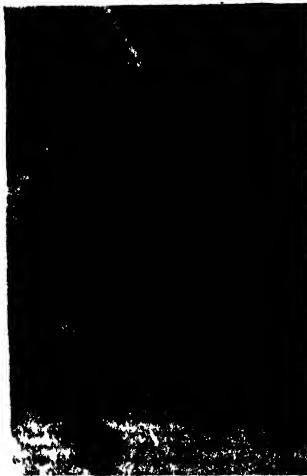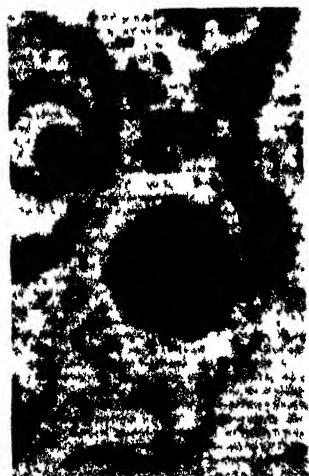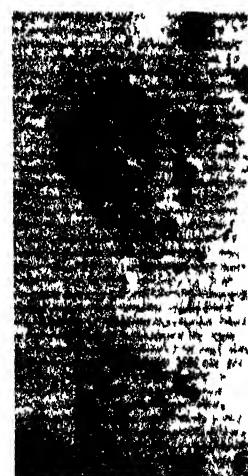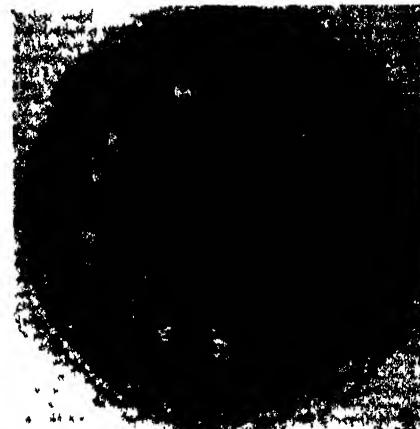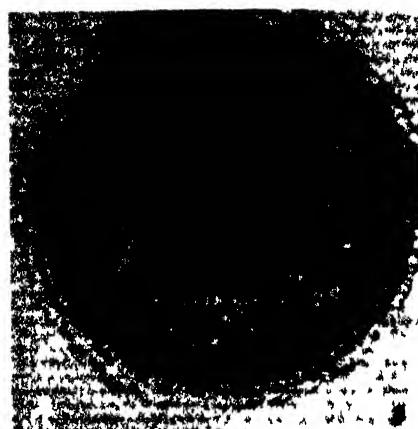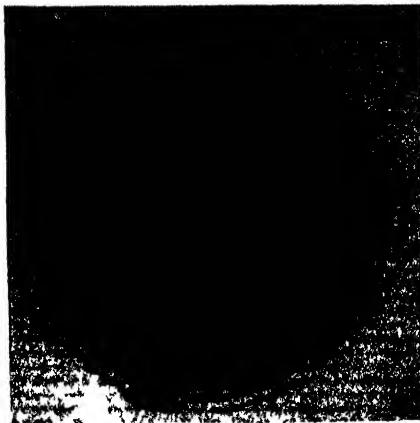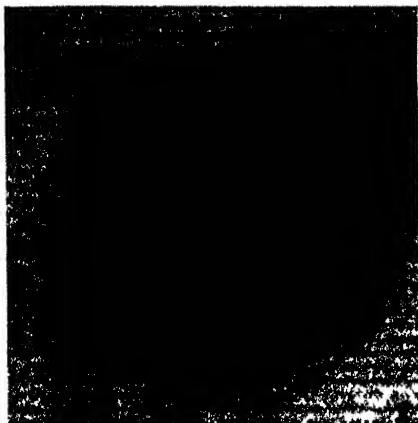
chromatids were loosely held together at the SA-region as they approached the metaphase plate. This same phenomenon was also observed in tetraploid cells (figs. 17, 18). This would again indicate that this phenomenon is characteristic of *Kochia trichophylla*.

DISCUSSION

Various methods have been proposed to explain the origin of polysomatic cells. Breslawetz (1926), Huskins and Smith (1932), Meurman (1933), and Wulff (1936) suggested that polyploidy was due to a type of cell or nuclear fusion. A second hypothesis was given by Stomps (1910), de Litardière (1923), and Hardh (1939), in which the chromosomes undergo a double longitudinal split during the prophase stage. Wulff (1940) explained the doubling in the *Aizoaceae* by way of a "double longitudinal splitting" or "inner division," and Lorz (1937) stated that the chromosomes divide and separate at a faster rate than the nucleus as a whole.

The foregoing observations on *Atriplex patula, Chenopodium album,* and *Kochia trichophylla* further substantiate the evidence of Gentcheff and Gustafsson (1939), Berger (1941), and Ervin (1941), that polysomaty arises by a double chromosome reproduction during the resting stage. The paired, relationally coiled chromosomes were clearly visible at very early prophase, which signifies that the double reduplication had occurred somewhat before this time and therefore during the resting stage.

In *Spinacia* the sequence of polyploid cells is as follows: 2n unpaired—4n paired—4n unpaired—8n paired—8n unpaired—16n paired—and rarely 16n unpaired. In the species here reported on it is thought that as soon as the periblem cells have begun to enlarge all of the outer periblem cells become tetraploid. If these cells undergo division, the first mitosis after double reduplication will give prophase and metaphase stages with the chromosomes rather closely associated. Any division following this will have chromosomes which are relatively less associated, the pairing being disturbed by the first mitosis.

There are undoubtedly more octoploid cells present in the root tips of *Atriplex patula* than were observed in division, but since these cells become

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Explanation of figures 8–18

FIGS. 8 and 9 from iron-alum haematoxylin sections. FIGS. 10–18 from aceto-orcein smear preparations. Magnification of all figures × 1500. FIGS. 8–11 from *Atriplex patula*. FIG. 8, Paired octoploid metaphase periblem cell. FIG. 9, Paired octoploid late prophase cell of the periblem. FIG. 10, Paired tetraploid early prophase stage. FIG. 11, Paired tetraploid early prophase stage (somewhat earlier than fig. 10). FIG. 12, Tetraploid early prophase stage of *Chenopodium album*. FIG. 13, Paired tetraploid early prophase stage of *Kochia trichophylla*. FIG. 14, Paired tetraploid metaphase stage of *Atriplex patula*. FIG. 15, Paired tetraploid anaphase of *Atriplex patula*. FIG. 16, Diploid metaphase plate of *Kochia trichophylla* with chromatids separated. FIGS. 17, 18, Tetraploid metaphase plates of *Kochia trichophylla*.
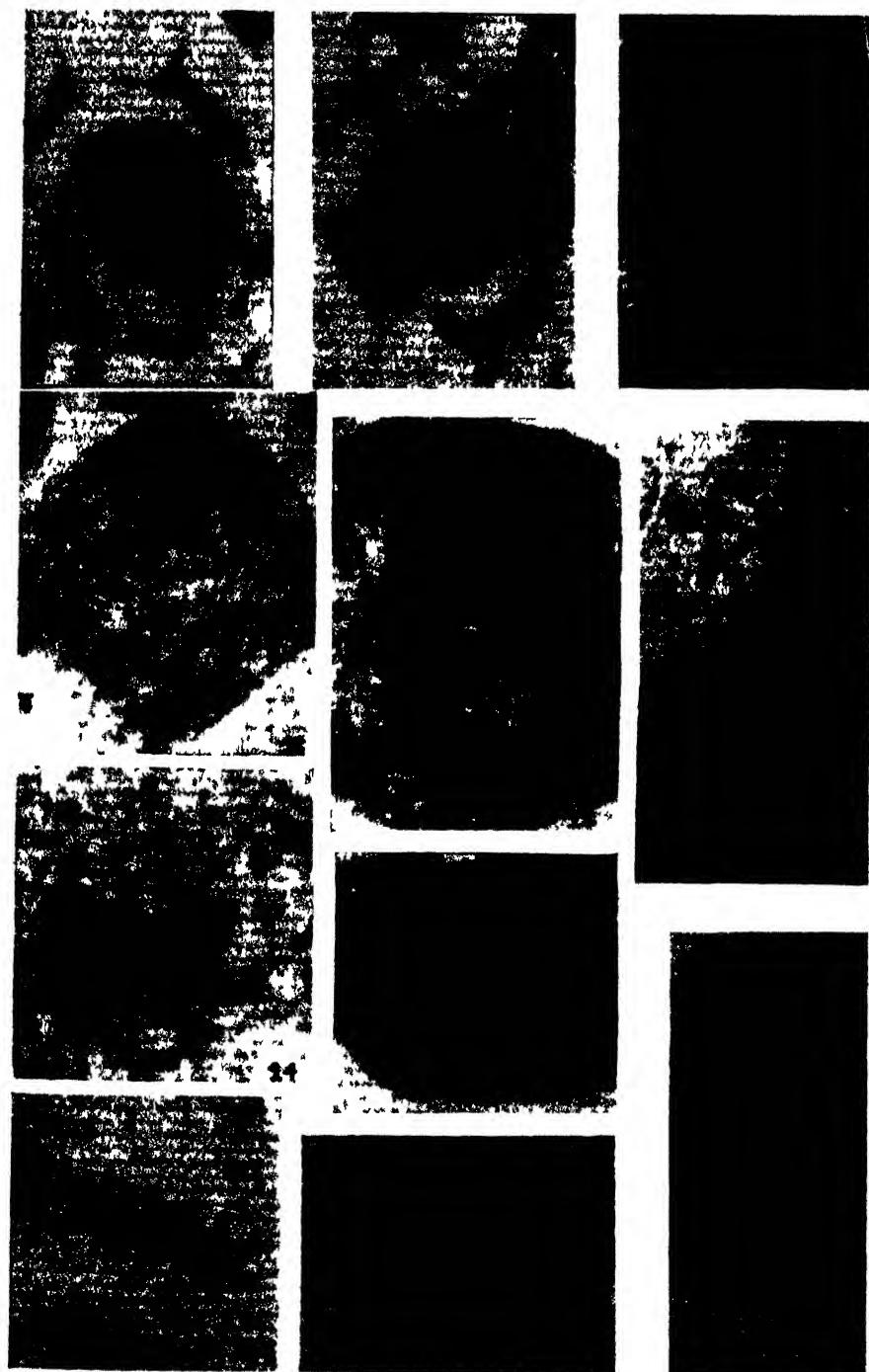
polyploid only after a considerable degree of differentiation has occurred very few of them undergo division. Since only paired octoploids occurred, it can be concluded that these cells become differentiated before another division can occur. While no octoploid divisions were observed in either *Chenopodium album* or *Kochia trichophylla* this does not denote a complete absence of such cells. Wulff (1936) investigated *Kochia scoparia* and *Kochia trichophylla* and found tetraploid cells with 36 chromosomes and octoploids with 72.

Since the meristematic region is shorter in *Chenopodium album* and *Kochia trichophylla* than in *Atriplex patula* it seems very possible that the degree of polyploidy reached in a particular species might be related to the length of the meristematic region. If the region of active division is very short then it would be expected that the cells would not have sufficient time to undergo many double reduplications. In *Chenopodium album* and *Kochia trichophylla* no octoploid cells were observed. These two species have a short meristematic region and the cells differentiate before they have an opportunity to become octoploid. In *Atriplex patula*, with its longer meristematic region, some cells have sufficient time to undergo a second double reduplication. In *Spinacia* the long meristematic region offers a good explanation of the high degree of polyploidy attained.

This interpretation not only explains the different degrees of polysomaty found in different species but offers a useful suggestion for understanding the difference between polysomatic and non-polysomatic species. It is quite possible that many supposedly non-polysomatic species form tetraploid cells which differentiate before they have an opportunity to reveal their polyploid condition by mitosis.

In *Atriplex patula* and *Chenopodium album* polyploid cells were observed not only in the periblem region but also in the dermatogen. Polysomaty occurred only in the periblem of *Kochia trichophylla*. Ervin's (1941) statement, that he considered the condition in *Cucumis melo* as apparently similar to that in various members of the *Chenopodiaceae* and that the main difference appeared to be that in the *Chenopodiaceae* the dermatogen was wholly monosomatic whereas in *Cucumis melo* some polysomatic cells were usually present in the dermatogen, needs, therefore, to be modified slightly. At least in some members of the *Chenopodiaceae* the dermatogen is not entirely monosomatic.

Ervin (1941) reported a paired condition of chromosomes during anaphase movement similar to that observed occasionally in *Atriplex patula*. In these cases of prolonged association the pairing is lost during later anaphase and telophase so that at the next division the chromosomes are unpaired.

Wulff (1936) remarked that in the genus *Chenopodium*, the polyploid species, with the exception of *C. Bonus-Henricus*, showed polysomaty; the

diploid species did not show this phenomenon. Since 36 chromosomes were determined as the diploid number for *C. album*, this plant is a polyploid species. As this species contains polysomatic tissue it thus supports Wulff's conclusion that only polyploid species of *Chenopodium* show polysomaty.

The separation of chromatids prior to their alignment on the metaphase plate as observed in *Kochia trichophylla* might perhaps be explained by a special condition of the matrix. In *Kochia trichophylla* the matrix apparently loses its ability to hold the chromatids together much earlier than it does in the majority of plants, where the separation does not occur until the chromosomes are at the equatorial plate, and where separation begins at the SA-region and then progresses terminally. It is generally known that chromatids usually come to lie even closer together at metaphase, so that they frequently appear as a single structure.

A phenomenon very similar to the early separation of chromatids described here has been reported by Grell (1945, 1946) in *Culex pipiens*. In *Culex* Grell has described four types of prometaphase chromosomes in which a more or less continuous series ranged from a condition in which the SA-regions were undivided and chromatid arms were partially or completely in contact to one in which both SA-regions and chromatid arms were completely separated and lay parallel to each other. That these paired, parallel bodies were actually chromatids was demonstrated by their subsequent behavior; the members of each such pair went to opposite poles at anaphase. According to Grell's classification the prometaphase chromosomes of *Kochia trichophylla* could be considered belonging to the fourth type.

SUMMARY

1. The root structure of *Atriplex patula*, *Chenopodium album*, and *Kochia trichophylla* is described. The meristematic region in *Atriplex patula* is slightly longer than the same region in *Chenopodium album* and *Kochia trichophylla*.

2. Diploid cells of *Atriplex patula* contain 18 chromosomes while diploid cells of *Chenopodium album* have 36 chromosomes. The chromosome number of 18 found in *Kochia trichophylla* agrees with previous reports.

3. Polysomaty occurs in the periblem and dermatogen of *Atriplex patula* and *Chenopodium album* and in the periblem of *Kochia trichophylla*.

4. Polysomaty in the three plants investigated, is brought about by a double reduplication in the resting stage, as was earlier reported for *Spinacia oleracea*.

5. The inner periblem cells are always diploid, while polyploid cells are present in the outer layer of the periblem.

6. The proportionately large number of early paired prophase stages as compared with the number of later prophase and metaphase stages indicates a prolonged early prophase condition in tetraploid cells.

7. It is suggested that the length of the meristematic region may be a factor in determining the degree of polyploidy reached. *Atriplex patula,* having a slightly longer meristematic region, contains some octoploid cells.

8. The chromatids in *Kochia trichophylla* separate before reaching the metaphase plate. This condition is observed in diploid and tetraploid cells.

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SOMATIC CHROMOSOMES OF PEDIGREED HYBRID PETUNIA

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INTRODUCTION

In the spring of 1946 the author was offered the opportunity of studying the somatic chromosomal complements of the members of a pedigreed hybrid population of *Petunia*.[2] Genetical breeding experiments on this population have been in progress for fifteen years with a view to determine the various types, nature, genetics, and effects of the different classes of both intraspecific and interspecific sterilities.

The present report is limited mainly to the determination of the chromosome numbers occurring in the progenies of 4n *Petunia axillaris* × 2n *P. axillaris* and of 4n *P. axillaris* × 2n *P. integrifolia*. The analysis of these progenies from the viewpoint of the specialized factors operating in fertility and sterility and the description of the morphology and growth habits lie within the scope of a forthcoming publication of Dr. A. B. Stout.

The present report has certain definite advantages over preceding reports of chromosome numbers in progenies of crosses in *Petunia*. The progenies here reported upon were derived from crosses of two pure species of *Petunia* and the range of crosses embraces higher-polyploid forms than had been previously reported for this genus.

The reports on naturally occurring polyploid races in the plant kingdom, on the experimental production of polyploid forms and on crosses involving polyploids have produced a vast amount of literature. The present paper is a contribution to the general problem of polyploidy and serves more particularly as a cytological basis for the interpretation of fertilities and sterilities in *Petunia*.

MATERIAL AND METHODS

Primary Species of the Population of Petunia. *Petunia integrifolia* (Hook.) Schinz & Thellung (1915) has small "violet" flowers and is very different from the two other primary species described below. Every individual of this species is self-incompatible. The use of the binomial *P. integ-*

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rifolia in preference to *P. violacea* is in accordance with the present rules of international nomenclature according to the findings of Stout (personal communication).

P. axillaris (Lam.) B.S.P. (*P. nyctaginiflora* Juss.) is very distinct, with white flowers and other clearly differentiated characteristics. Every individual plant of the species is self-incompatible according to Stout and Chandler (1941, 1942) and Stout (1945).

P. parodii Steere (1931) is somewhat similar to *P. axillaris* but quite distinct and always fully self-compatible.

Tetraploid Forms of P. axillaris. Diploid plants of *P. axillaris* were treated with colchicine by Stout and Chandler, (1941, 1942) and a number of tetraploid forms were obtained.

Tetraploid-diploid Crosses and Progenies. All the progenies studied in this work were derived from the two crosses, 4n *P. axillaris* × 2n *P. integrifolia*, and 4n *P. axillaris* × 2n *P. axillaris*. The progenies are listed below as series and the parentages are indicated with the seed parent listed first in each cross.

Series 170. The parents of this series are 4n *P. axillaris* × 2n *P. integrifolia*.

Series 170–10. This is a clone derived from a branch of 170–4 which had been treated with colchicine. Each member of the clone is termed a *ramet* (Stout 1929). Ten ramets were examined.

Series 217. The parents are two ramets of 170–10.

Series 224. The parents are two ramets of 170–10.

Series 227. The parents are two ramets of 170–10.

Series 245. The parents are 139–12T 4n *P. axillaris* × 2n *P. axillaris*.

Series 246. The parents are 172–28 4n *P. axillaris* × 2n *P. axillaris*.

Series 247. The parents are 139–12T 4n *P. axillaris* × 2n *P. axillaris*.

Series 235. The parents are 170–3 × 2n *P. integrifolia*.

Series 238 and 239. The parents are 170–1 × 2n *P. integrifolia*.

Series 242. The parents are 170–4 × 139–12 2n *P. axillaris*.

Series 243. The parents are 170–5 × 139–12 2n *P. axillaris*.

Series 230. The parents are 170–3 × 170–1.

Series 318. The parents are 170–3 × 170–5.

Series 319. The parents are 170–7 × 170–1.

Series 320. The parents are 170–4 × 170–10.

Series 321. The parents are 170–10 × 170–10.

Series 241. The parents are 170–7 × 139–12T 4n *P. axillaris*.

Series 266. The parents are 170–5 × 171–28 4n *P. axillaris*.

Series 279. This series includes the progeny from the selfing of 217–4.

Series 280. This series includes the progeny from the selfing of 217–5.

Series 281. This series includes the progeny from the selfing of 217–18.

Series 282. This series includes the progeny from the selfing of 224–5.

Series 283. This series includes the progeny from the selfing of 227–2.

Series 284. This series includes the progeny from the selfing of 224–1.

Series 285. The parents are 217–4 × 139–12T 4n *P. axillaris*.

Series 286. The parents are 217–12 × 171–28 4n *P. axillaris*.

Series 287. The parents are 222–6 4n *P. axillaris* × 224–5.

Cytological Methods. The examination of the chromosomal complements of the various members of the population of *Petunia* was made by the

leaf smear technique of Baldwin (1939) in union with the Feulgen stain
(Meyer 1943). The fixation period was 1–2 hours in 3:1 absolute alcohol–
glacial acetic acid. The young leaves surrounding a young vigorous flower
bud were found most suitable. After fixation, the young leaves were rinsed in
tap water for 1–5 minutes and then hydrolyzed in N HCl at 55–60° C for
10 minutes. The hydrolyzed leaves were rinsed in tap water for 1–5 minutes
and then placed in a vial of decolorized fuchsin for 20–30 minutes. The
entire stained leaves were then placed in 45 % acetic acid and the basal third
of the leaf, in which most mitotic activity is present according to Speese
(1939), was cut into pieces of approximately 0.5 mm. The use of 10 %
acetic in place of the 45 % acetic was found to allow greater pressure in the
subsequent smearing. Forceful pressure by means of a matchstick on the
cover glass produced flat, well spread figures.

The temporary smears were made permanent according to the method
of McClintock (1929) with the following modifications: the temporary
smears were left overnight in a moist chamber containing equal parts of
45 % acetic acid and 95 % alcohol, the fluid barely touching the lower edge
of the cover slip. On the following day a razor blade was gently inserted
beneath the edge of the cover slip and the slip lifted off. The smear almost
invariably adhered to the cover slip. The cover slip was then run through
two changes of 95 % alcohol and combined with a new slide in Diaphane. A
counter-stain of orange G or light green was frequently added in the first
95 % alcohol.

Pretreatments. All the leaf smears were pretreated. Burrell (1939) had
suggested the use of colchicine to contract chromosomes as a pretreatment
for root-tip smears. Meyer (1943) used this colchicine pretreatment in con-
junction with the leaf smear technique and Feulgen stain. The scarcity of
colchicine forced the author to use a variety of substitutes in the pretreat-
ment of the leaf smears.

Several substitutes employed by the author had been reported previously
in the literature, namely, paradichlorobenzene (Meyer 1945), acenaphthene
(Swanson 1940), and chloral hydrate, which had had a long history since
the work of Němec (1904) and has been employed in particular by Swan-
son (1943).

Pretreatment with p-dichlorobenzene was relatively unsuccessful al-
though many attempts were made. There was generally only a very slight
contraction of the chromosomes. In some cases contraction did occur and it
was possible to spread the chromosomes but the chromosomes lacked clear cut
outlines in these smears (fig. 12). The results with acenaphthene were
similar except that the chromosomes had somewhat clearer outlines.

The action of chloral hydrate was so toxic that it was not found possible
to obtain any accurate counts of chromosomes. The abnormal condition of the

excised leaves may have accentuated the toxic effects. Dividing cells were very rare and contraction, when it was observed, showed the chromosomes as mere "blocks."

In the fall of 1946, Dr. H. Wechsler brought to the attention of the author a reference to the work of King and Sullivan (1946) on the similarity of action of podophyllin and colchicine. The present author undertook a rapid test of the action of podophyllin on excised root tips of *Allium cepa* and on young excised leaves of *Petunia*. Sullivan and Wechsler (1947) reported the c-mitotic activity of podophyllin on growing root tips of *Allium cepa*.

The pretreatment with podophyllin consisted of 1 g. of podophyllin in 100 ml. of tap water, thoroughly shaken and allowed to stand overnight. The mixture was filtered and then used for the pretreatment of the young excised leaves.

The contracting effect of podophyllin alone was very similar to that of p-dichlorobenzene and acenaphthene but the number of dividing cells seemed much greater after the pretreatment with podophyllin. The degree of contraction was very slight even after prolonged pretreatment and so this agent alone did not prove to be of great value in counting small chromosomes. Some good preparations were obtained (figs. 15, 16).

The pretreatment with podophyllin followed by chloral hydrate produced many good figures that spread well. The most useful combination was a 2-hour pretreatment of podophyllin followed by 3 hours of a 0.06 % chloral

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Explanation of figures 1–15

FIGS. 1–15. Photomicrographs of *Petunia* leaf smears. Magnification × 2352. FIG. 1. Feulgen leaf smear of diploid (14) *P. axillaris*. Pretreatment: 2 hours of 1% podophyllin followed by 3 hours of 0.06% chloral hydrate; this same pretreatment used on material shown in figures 2, 3, 4, 5, and 6. FIG. 2. Aceto-orcein leaf smear of diploid (14) *P. integrifolia*. FIG. 3. Aceto-orcein leaf smear of diploid (14) *P. parodii*. FIG. 4. Feulgen leaf smear of 4n (28) *P. axillaris*. FIG. 5. Feulgen leaf smear of a triploid (21) from a cross 4n *P. axillaris* × 2n *P. integrifolia*. This plant was the source of the colchicine induced hexaploids. FIG. 6. Feulgen leaf smear of a hypotriploid (20) segregate from a cross 4n *P. axillaris* × 2n *P. integrifolia*. FIG. 7. Feulgen leaf smear of a diploid (14) segregate from a cross of hybrid 3n (21) × 2n *P. axillaris*. Pretreatment: 3 hours of a solution containing 0.5 g. podophyllin and 0.05 g. chloral hydrate. The same pretreatment was used on material shown in figures 8, 10, and 13. FIG. 8. Feulgen leaf smear of a trisomic (15) segregate from a cross of hybrid 3n (21) × 2n *P. axillaris*. FIG. 9. Feulgen leaf smear of a polysomic (16) segregate from a cross of a hybrid 3n (21) × 2n *P. integrifolia*. Pretreatment: 1.5 hours of podophyllin followed by 1.5 hours of 0.1% chloral hydrate. This same pretreatment was used on material shown in figures 11 and 14. FIG. 10. Feulgen leaf smear of a polysomic (16) segregate from a cross of hybrid 3n (21) × 2n *P. axillaris*. FIG. 11. Feulgen leaf smear of a polysomic (17) segregate from a cross of hybrid 3n (21) × 2n *P. integrifolia*. FIG. 12. Feulgen leaf smear of a polysomic (18) segregate from a cross of hybrid 3n (21) × 2n *P. integrifolia*. FIG. 13. Feulgen leaf smear of a polysomic (19) containing a fragmented chromosome from a cross of hybrid 3n (21) × 2n *P. axillaris*. FIG. 14. Feulgen leaf smear of a hypotriploid (20) segregate from a cross of hybrid 3n (21) × 4n (28) *P. axillaris*. FIG. 15. Feulgen leaf smear of a polysomic (19) segregate from a cross of two hybrid triploids. Pretreatment: 3 hours of podophyllin.
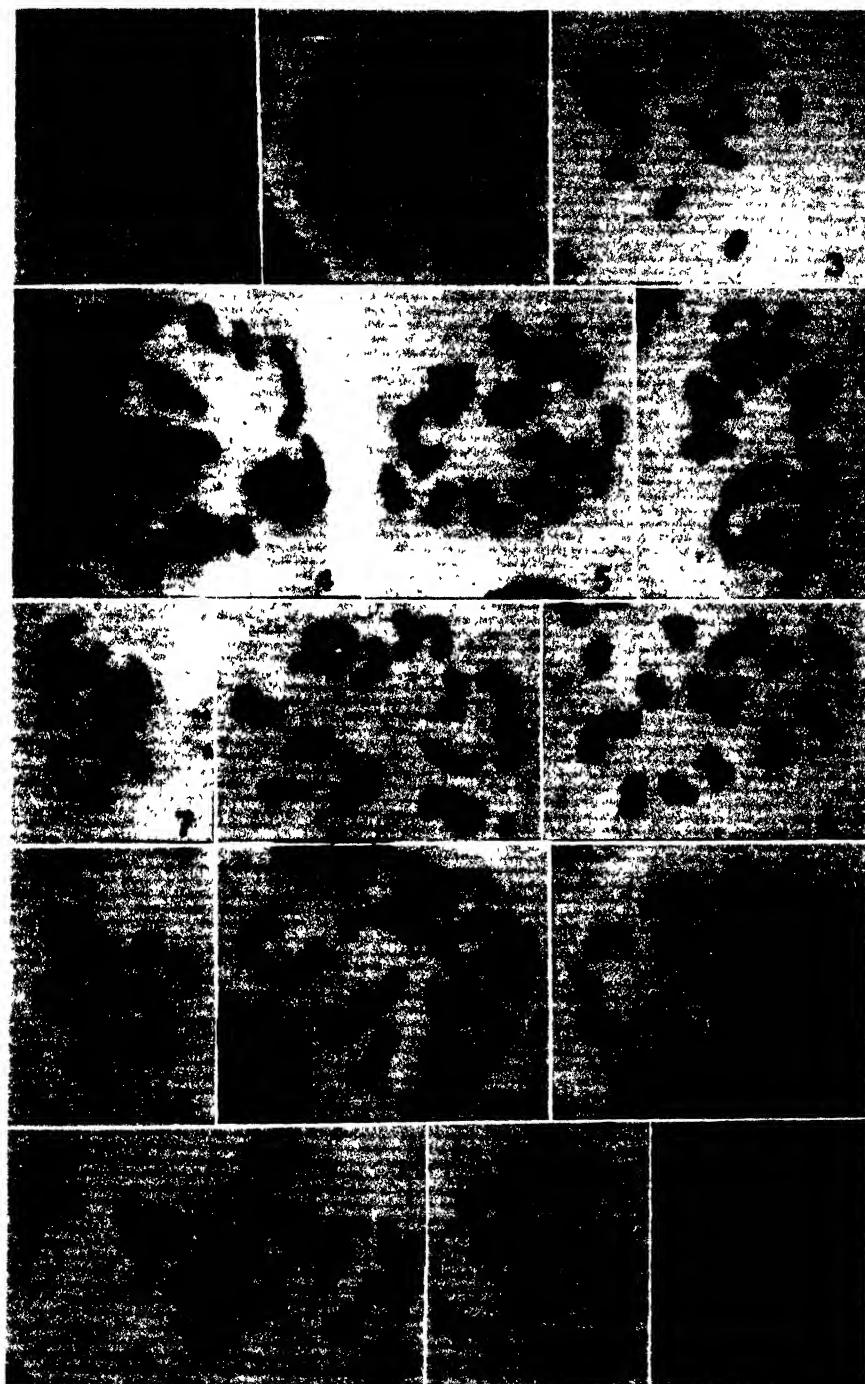
hydrate (figs. 1, 2, 3, 5, 6, 20). Good results were also obtained using 1.5 hours of podophyllin followed by 1.5 hours of a solution containing 0.1 % chloral hydrate (figs. 4, 14, 27, 28). The single solution containing 0.5 g. podophyllin and 0.05 % chloral hydrate was used with good success in a pretreatment period of 3–4 hours (figs. 4, 8, 10, 13, 17, 21, 23, 25).

In particular, the following pretreatments were used: (1) a 0.05 % aqueous solution of colchicine for 3–4 hours; (2) a saturated aqueous solution of p-dichlorobenzene prepared the night before and left overnight at a constant oven temperature of 55–60° C and used as a pretreatment for 1.5 hours; (3) a saturated aqueous solution of acenenaphthene for 3.5 hours; (4) the filtrate of 1 g. of podophyllin in 100 ml. of tap water for 1.5 to 3 hours; (5) the filtrate of 1 g. of podophyllin in 100 ml. of tap water for 1.5 hours followed by a second pretreatment of 1.5–3 hours of an aqueous solution containing either 0.06 % or 0.1 % of chloral hydrate; (6) a single solution containing 0.5 g. podophyllin and 0.05 g. chloral hydrate with or without 0.06 % $NaHCO_3$.

Aceto-orcein smears.   The aceto-orcein smear method of LaCour (1941) had been used in the leaf smear technique by Rösen (1946). The pretreatments mentioned above were used by the present author in conjunction with the aceto-orcein smear method in a few instances.

The counts were made by observation of permanent smears with the aid of a fluorite oil-immersion lens of a power of 98× in conjunction with an ocular of 15×. All the photomicrographs except one (fig. 26) were made with a Leitz "Makam" camera of magnification of 1× using an 8× ocular and a Wratten M filter no. 58.

<div align="center">OBSERVATIONS AND RESULTS</div>

Morphology of Chromosomes.   The length of the untreated metaphase chromosomes in all three primary species is quite similar and has a range from 2.5 to 4.5μ. The width is approximately 0.6μ. The only observation of a SAT-chromosome in the primary diploid species was in *P. axillaris.* It is difficult to observe the SAT-chromosome as the satellited region is generally hidden and is quite minute.

The measurements of treated chromosomes were made in 52 different plant smears that represent plants from all the types of crosses reported upon. The range of length and breadth of the various degrees of contraction extended from $5.4 \times 0.6\mu$ to $1.26 \times 1.0\mu$. It is easily seen that many of the figures measured were prophases. Figure 11 represents a prophase stage, as can be seen from the uncontracted chromosomes, and this may be the reason for the clarity of the SAT-chromosome seen there. The measurements of chromosomes in the various polyploid forms seem to indicate no change in size but since the chromosomes are quite small a slight change in size might go unobserved. The fragment seen in figure 13 measured $0.6 \times 0.6\mu$.

Somatic Chromosome Numbers. The diploid number of all species of *Petunia* has been reported as 14 except for the species *P. parviflora* which has the number 18 (Ferguson & Coolidge 1932; Steere 1932). Ferguson (1924) reported the somatic count of 14 for diploid bedding petunias and the same number was reported for true breeding *P. violacea* Lindl. (Ferguson & Coolidge 1932). Numerous reports of the diploid number of 14 for varieties classified as *P. violacea* have been made and are listed by Ferguson and Coolidge. Ferguson (1928) reported the diploid number of 14 for pure *P. axillaris* and this was confirmed by Steere (1932). The diploid number of 14 was reported by Steere (1931) for *P. parodii* in his description and determination of that new species of *Petunia*. The present author confirms the counts for the three species. Unexpectedly, a fragment was observed in one of the diploids of *P. parodii*. The chromosome complements of the three primary species are seen in figures 1, 2, and 3.

Tetraploid P. axillaris. Seven determinations of the originally induced tetraploids and their progeny were made. All plants were exact tetraploids. This is a confirmation of the more extensive meiotic counts of Stout and Chandler (1941). One tetraploid complement is seen in figure 4.

Segregates from 4n P. axillaris × 2n P. integrifolia. All seven segregates were determined as exact triploids with a count of 21, except plant no. 170–6 which is seen in figure 6 with a complement of 20 chromosomes. A typical count of the triploid hybrids is seen in figure 5.

A colchicine-treated triploid. The triploid hybrid 170–4 was treated with colchicine in order to induce the hexaploid condition. The only ramets available to the author were basal cuttings, which were unaffected by the colchicine treatment. There is no doubt from the evidence given below that the upper cuttings had been changed to the hexaploid condition. It was to be expected that part of the treated plant might remain unaffected by the treatment from the work of Blakeslee and Warmke (1940).

Tetraploid P. axillaris × 2n P. axillaris. Four plants were selected at random from the progeny of this cross and all were determined to be exact triploids (21).

Hybrid triploid 170–3 × 2n P. integrifolia. Only the classes 15 (trisomic), 16, and 17 were found in the progeny of this 3n × 2n cross (table 1). Typical counts are seen in figures 9 and 11.

Hybrid triploid 170–1 × 2n P. integrifolia. All the chromosome classes from diploid to hypotriploid (20) were found in the progeny of this 3n × 2n cross (table 1). A photomicrograph of a typical count is seen in figure 12.

Hybrid triploid 170–4 × 2n P. axillaris. All chromosome classes from 15 (trisomic) to 20 were found in the progeny of this 3n × 2n cross. Typical counts are seen in figures 8, 10, and 13. A fragment can be seen in figure 13.

Hybrid triploid 170–5 × 2n P. axillaris. The chromosome classes from

diploid (14) to 20 with the exception of classes 17 and 18 were found in the progeny of this 3n × 2n cross. Figure 7 shows a diploid (14) segregate from this cross.

TABLE 1. *Chromosome counts from pedigreed 3n × 2n crosses.*

Seed parent	Pollen parent	Chromosome numbers								Total
		14	15	16	17	18	19	20	21	
170–3(3n) × 2n	*P. integrifolia*		4	6	2					12
170–1(3n) × 2n	*P. integrifolia*	1	6	1	1	3	1	3		16
170–5(3n) × 2n	*P. axillaris*	3	1	1			1	1		7
170–4(3n) × 2n	*P. axillaris*		1	1	1	2	1f[a]	4		10

[a] Fragment.

Hybrid triploid crosses. The specific triploid-triploid crosses and the somatic chromosome determinations of the members in their progenies are listed in table 2. Of the 10 chromosome classes from 19 to 28 (tetraploid), 7 chromosome classes are represented. There is an apparent concentration in the chromosome class 20. The chromosome classes 19, 20, and 23 have not hitherto been reported for triploid crosses in *Petunia*. Counts of the classes 19 and 25 are seen in figures 15 and 16 respectively.

Triploid-tetraploid crosses. The cross of 170–5 (3n) × 4n *P. axillaris* yielded only one plant, with the count of 20 chromosomes. A similar cross of 170–7 (3n) × 4n *P. axillaris* also yielded only one plant, which had a count of 26 chromosomes (fig. 17).

Explanation of figures 16–25

FIGS. 16–25. Photomicrographs of Feulgen leaf smears of *Petunia*. Magnification × 2352 unless otherwise noted. FIG. 16. Feulgen leaf smear of a polysomic (25) segregate from a cross of two hybrid triploids. Pretreatment: 3 hours of 1% podophyllin. FIG. 17. Feulgen leaf smear of a polysomic (26) segregate from a cross of hybrid triploid (21) × 4n (28) *P. axillaris*. Pretreatment: 3.5 hours of a solution containing 0.5 g. podophyllin and 0.05% chloral hydrate. The same pretreatment was used on material shown in figures 21, 23, and 25. FIG. 18. Feulgen leaf smear of a hypertetraploid segregate (29) from a cross of hybrid hexaploid (42) × 4n (28) *P. axillaris*. Pretreatment: 1.5 hours of podophyllin followed by 1.5 hours of 0.1% chloral hydrate. The same pretreatment was also used on material shown in figures 19, 22, and 24. FIG. 19. Feulgen leaf smear of a polysomic (31) segregate from a hybrid hypohexaploid (41) × 4n (28) *P. axillaris*. FIG. 20. Feulgen leaf smear of a polysomic (32) segregate from a cross of a hybrid hexaploid (42) × 4n (28) *P. axillaris*. Pretreatment: 2 hours of 1% podophyllin followed by 3 hours of 0.06% chloral hydrate. FIG. 21. Feulgen leaf smear of a polysomic (33) segregate from a cross of a hybrid hypohexaploid (41) × 4n (28) *P. axillaris*. Magnification × 1715. FIG. 22. Feulgen leaf smear of a polysomic (34) segregate from a cross of a hybrid hexaploid (42) × 4n *P. axillaris*. FIG. 23. Feulgen leaf smear of a pentaploid (35) segregate from a cross of a hybrid hexaploid (42) × 4n (28) *P. axillaris*. FIG. 24. Feulgen leaf smear of a hyperpentaploid (36) segregate from a cross of a hybrid hexaploid (42) × 4n (28) *P. axillaris*. FIG. 25. Feulgen leaf smear of a hypohexaploid (40) segregate from the selfing of an colchicine-induced hexaploid.

Hexaploid progenies. The hybrid triploid 170–4 had been subjected to colchicine treatment to induce hexaploidy (Stout, personal communication). Unfortunately, none of the original cuttings was available to the author for
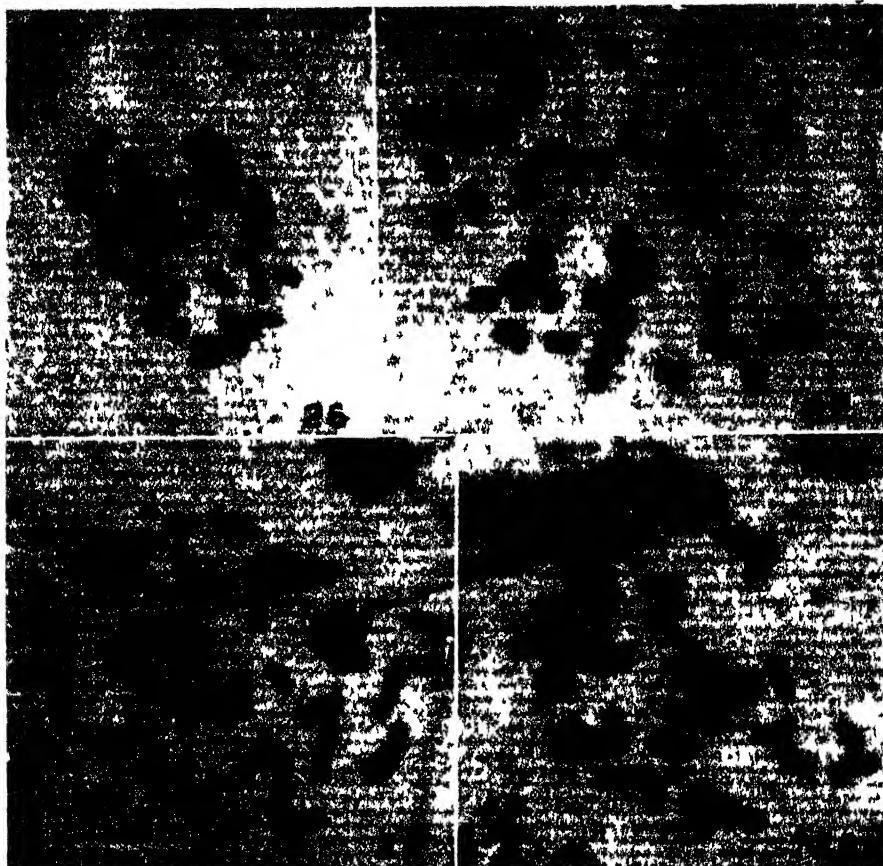
FIGS. 26–29. Photomicrographs of Feulgen leaf smears of *Petunia*. Magnification × 2352 unless otherwise noted. FIG. 26. Feulgen leaf smear of a hypohexaploid (41) segregate from the selfing of a colchicine induced hybrid hexaploid. Pretreatment: 2 hours of 0.06% colchicine. Magnification × 1568. FIG. 27. Feulgen leaf smear of a balanced hexaploid (42) segregate from the selfing of a colchicine induced hexaploid. Pretreatment: 1.5 hours of 1% podophyllin followed by 1.5 hours of 0.1% chloral hydrate. The same pretreatment was also used in the material shown in figure 28. FIG. 28. Feulgen leaf smear of a hyperhexaploid (43) segregate from the selfing of a hybrid hexaploid. FIG. 29 Feulgen leaf smear of a hyperhexaploid (44) segregate from hybrid hypohexaploid (42) × 4n (28) *P. axillaris*. Pretreatment: 1.5 hours of 1% podophyllin followed by 1.5 hours of 0.1% chloral hydrate.

determination. However, the plants grown from these treated cuttings were selfed and 25 plants were examined for chromosome counts: 16 were exact hexaploids, 6 had 40 chromosomes, and 3 fell into the 41 class (table 3).

TABLE 2. *Chromosome counts from pedigreed 3n × 3n crosses.*

Seed parent Pollen parent	Chromosome numbers									
	19	20	21	22	23	24	25	26	27	28
170–3 (3n) × 170–1 (3n)	1	3					1			
170–3 (3n) × 170–5 (3n)					1					
170–7 (3n) × 170–1 (3n)		1				1	2			
170–4 (3n) × 170–10(3n)								1		
170–10(3n) × 170–10(3n)					1					1
	1	4			2	1	3	1		1

These derived hexaploids were in turn selfed and a large progeny was obtained. Fifty plants were examined: 26 plants had exact hexaploid complements (42), 10 were in the 40 chromosome class, 7 were in the 43 class, 6 in the 42 class, and 1 in the 44 class (table 3). Typical complements of the chromosome classes 40 to 44 are seen in figures 25–29 respectively.

6n × 4n crosses and the reciprocal.   The cross of 217–4 (41) × 4n *P. axillaris* yielded a large progeny, of which 16 plants were examined at random. The chromosome classes from 29 to 35 were represented with the exception of class 30 (table 4). The similar cross of 217–4 (41) hybrid hypohexaploid × 4n *P. axillaris* also yielded a large progeny, of which 16 plants were examined and all found to fall within the chromosome classes from 32 to 36 (table 4). The percentages of distribution are also included in table 4 and show that there is some factor acting to produce a higher range in the second cross. The reciprocal of the above crosses is found in 4n *P. axillaris* × hybrid hexaploid 224–5, which yielded only one plant, with the chromosome count of 35. Typical complements of the chromosome classes in the progenies of 6n × 4n crosses are seen in figures 18–24.

TABLE 3. *Chromosome class distribution of hexaploid progenies.*
The lower number in each generation is the percentage of the total.

Chromosome classes	40	41	42	43	44	Total
C–1 generation	6	3	16			25
	24	12	64			
C–2 generation	10	6	26	7	1	50
	20	12	52	14	2	75

DISCUSSION

Genetical Purity of the Primary Species.   While reference to and comparison with previous reports of experimental crosses within the genus *Petunia* will be made below, it should be emphasized that the crosses reported upon in this paper were made with two genetically pure species,

TABLE 4. *Chromosome class distribution in the progenies of hybrid hexaploid* × *4n P. axillaris.*

Percentages of total plants falling within each chromosome class.

Chromosome class	Seed parent 217-4 (6n) (41)	Per cent	Seed parent 217-12 (6n)	Per cent
29	1	6.3		
31	3	18.7		
32	3	18.7	2	12.5
33	4	25.0	4	25.0
34	3	18.7	2	12.5
35	2	12.5	6	37.5
36	0	0.0	2	12.5

namely *P. axillaris* (Lam.) B.S.P. and *P. integrifolia* (Hook.) Schinz & Thellung. The population of *Petunia* based on these two species is unique in another respect in that the tetraploid female parent, 4n *P. axillaris,* is the first tetraploid form in the pure species *P. axillaris* (Stout & Chandler 1941; Stout 1945).

Ferguson and Ottley (1932) insist very strongly that in view of the frequent misuse in earlier investigations of the binomials *P. violacea* Lindl. and *P. nyctaginiflora* Juss., the gametic purity of the parents in any investigation should be determined at least for those characters which are to be studied in the succeeding generations. These authors refer more particularly to *P. violacea,* which they maintain with Steere (1932) is not found in a pure state in cultivation. Ferguson and Ottley (1932) cite evidence for the fact that the binomial *P. violacea* was used from 1836 onward as a "blanket tag" for small-flowered petunias grown in greenhouses and gardens and which were the hybrid varieties resulting from the crossing of the pure species *P. violacea* and *P. axillaris.* These pure species were imported to Europe from South America during the period 1823–1835.

The taxonomic history and specific characteristics of both *P. axillaris* and *P. violacea* are given by Ferguson and Ottley (1932). These binomials were retained by those authors after an analysis of the evidence for synonymy in these two species. The combinations *P. integrifolia* and *P. axillaris* have been used throughout this paper in view of recent detailed reinvestigation of synonymy in these two species by Stout (1947, written communication).

Previous reports of chromosome counts in the progenies of crosses in *Petunia* are found in the papers of Dermen (1931), Kostoff and Kendall (1931), Steere (1932), Matsuda (1935), Levan (1937), Stout and Chandler (1941, 1942) and Stout (1945). In the crosses reported before 1941, the only mention of a pure species used in a cross in *Petunia* is that of the diploid *P. axillaris* in the work of Steere (1932). There is a very notable difference between the diploid *P. axillaris* used by Stout and Chandler (1941, 1942) in

that the individual plants of Steere were self-fertile while those of Stout and Chandler were quite self-sterile. Stout (1947, personal communication) is of the opinion that the plants identified and figured as *P. axillaris* by Ferguson and Ottley (1932) and reported as 95 % self-fertile are not *P. axillaris*.

A Fragment in a Diploid P. parodii. This is apparently the first instance of a fragment reported in a pure diploid species of *Petunia*. Upcott (1936) cites the presence of such fragments in many plants and animals. A detailed study of such fragments occurring in diploids in *Tradescantia* has been made by Swanson (1943) but no certain conclusions could be drawn as to their origin. A sister plant of *P. parodii* did not contain the fragment (fig. 3).

Tetraploid P. axillaris. The somatic counts of the colchicine-induced tetraploid *P. axillaris* and the progeny of the selfed tetraploid are confirmations of the meiotic counts of Stout and Chandler (1941, 1942). It is to be noted that these authors reported the inactivation of the factors for incompatibility in the change from diploidy to tetraploidy (Stout & Chandler 1942; Stout 1945) and the hereditary transmission of induced tetraploidy and compatibility (Stout & Chandler 1942). All the determinations of these authors showed that there was general stability in the original tetraploid and its progeny inasmuch as all were exact tetraploids. Levan (1937) reported that of a progeny of 96 plants from a selfed 4n *P. violacea* 64 were exact tetraploids, 23 were in the hypotetraploid class with 27 chromosomes, 8 were hypertetraploid with 29 chromosomes, and one plant had 30 chromosomes. Matsuda (1935) reported that 29 of 31 plants in the progeny of a selfed tetraploid in *Petunia* were exact tetraploids. There is evidence given below that aneuploid gametes are formed in 4n *P. axillaris* but they do not apparently function in the selfing of the tetraploid. This would seem to be in agreement with the opinion of Müntzing (1931) and Levan (1942) that optimum viability exists when the egg and pollen have approximately the same chromosome number.

Tetraploid-Diploid Crosses. The segregates (22) from these crosses were all exact triploids with the exception of one plant which had the count of 20 and one with the count of 14. The cross 4n *P. axillaris* × 2n *P. axillaris* was reported earlier by Stout and Chandler (1942) as completely unsuccessful but Stout (personal communication) later found that the cross could be successful if it were between different genotypes.

There is a total of 129 counts for segregates from tetraploid-diploid crosses in *Petunia* in the combined reports of Kostoff and Kendall (1931), Steere (1932), Matsuda (1935), Levan (1937), and the present writer. The exact triploid class was found in 71.3 per cent of these 129 segregates. Mat-

suda (1935) is the only other investigator to report the exact reconstitution of the diploid from this type of cross. Diploid parthenogenesis and merogony are ascribed as the causes for the formation of these diploids. The tetraploids and near-tetraploids (hypotetraploids) reported by the above investigators are explained on the basis of fertilization and zygote formation from a diploid egg and diploid pollen grain (Matsuda 1935; Levan 1937). Hyper-tetraploids are due to diploid eggs with non-disjunctional chromosomes fertilized by diploid pollen (Matsuda 1935; Levan 1937).

The formation of triploids from the tetraploid-diploid cross is attributed to the union of a diploid egg and haploid pollen (Levan 1937). Experimental proof of such formation in *Crepis* is given by Navaschin (1929). It would be expected from the stability of the tetraploid *P. axillaris* that diploid eggs would be formed regularly. The zygotic viability of triploids with 2 genomes of *P. axillaris* and one of *P. integrifolia* is different from that of the triploid zygote containing 3 genomes of *P. axillaris* (Stout & Chandler 1942) and has been ascribed by Stout (personal communication) to the general rule that gametes containing similar factors for sterility, as in certain genotypes in the autotriploid zygote, are unviable.

Triploid-Diploid Crosses. The previous reports of the progenies of such crosses in *Petunia* are found in the reports of Matsuda (1935) and Levan (1937). Matsuda found that only 3 seeds out of 250 germinated and the plants on examination had the chromosome numbers 19, 20, and 21. This triploid (21) is the only report of such a segregate from a 3n × 2n cross in *Petunia*. The expectation of obtaining a triploid from such a cross is very slight, as can be seen from the comparative data for such crosses in several genera by Levan (1942). Table 5 contains the previous reports of chromosomal counts in the progenies of triploid-diploid crosses in *Petunia* in conjunction with the findings of the present author. There is complete agreement between the findings of Levan and those presented here in respect to the number of classes found' in the progeny. All the classes between 2n and 3n − 1 are found. The chromosome class with the greatest frequency is the trisomic (15), which includes 38.9 per cent of the segregates in Levan's material and 26.7 per cent in the author's material. If the classes 14, 15, and 16 are combined into one group, the value of this group in Levan's material is 85.5 per cent and 52.2 per cent in the material examined by the author. This heavy concentration in the diploid range is attributed to the non-random distribution in triploid meiosis that was noted early by Belling and Blakeslee (1922) and confirmed thereafter. In particular it may be noted that Satina and Blakeslee (1937b) found in triploid *Datura* in a study of the female gametophyte 30 times as many female gametes with n + 1 chromosomes and 280 times as many haploid gametes as would be expected if ran-

dom distribution were taking place. These authors attribute the high number of diploid and near-diploid progeny to chromosomal elimination of lagging chromosomes in both meiotic divisions.

There is however some disagreement between the distribution of chromosome classes found in the progenies of 3n × 2n crosses as reported by Levan (1937) and by the present author. Using the scheme of Levan (1942), the author has divided the 2n–3n range into five groups: group 14, group 15–16, group 17–18, group 19–20 and group 21 (table 5). As can be seen, the two sets of distribution diverge sharply in group 19–20 but there is a general tendency to the higher chromosome classes in the author's material. The group 19–20 is represented by only 3.3 per cent in Levan's material but accounts for 24.4 per cent in the progenies reported by the author. This percentage (24.4) is higher than any listed for 3n × 2n crosses in several

TABLE 5. *Distribution of chromosome classes in progenies of 3n × 2n crosses in Petunia.*

	Groups				
	I	II	III	IV	V
Chromosome classes	14 (2n)	15 16	17 18	19 20	21 (3n)
Number of plants				1 1	1 (Matsuda 1935)
Number of plants	18	35 24	5 5	2 1	0 Levan 1937)
Group %	20.0	65.5	11.1	3.3	0.0
Number of plants	4	12 9	4 5	3 8	0 (Sullivan, present paper)
Group %	8.8	46.7	20.0	24.4	0.0

genera in the review by Levan (1942). The highest figure given there for the comparable group (19–20) is 18.0 per cent for *Populus*, but this is considered to be a case of secondary polyploidy (Johnson 1942; Levan 1942). It may be that the triploid *Populus* resembles to some degree the hybrid triploid used in *Petunia* 3n × 2n crosses and reported upon here.

Differences of Specific 3n × 2n Crosses. The distribution of chromosome classes in the progenies of 3n × 2n crosses is different when the pollen parent *P. integrifolia* is substituted for *P. axillaris* (table 6). In addition there is considerable difference in the distribution depending on which 3n segregate is used as the seed parent in the cross (table 6). It seems that the 3n × 2n cross in which the pollen parent was *P. integrifolia* has a distribution that is much closer to that reported by Levan (1937) than the distribution of the 3n × 2n cross in which *P. axillaris* was the pollen parent. In the latter cross a most unusual distribution of the progeny tends to fall in the upper classes approaching the triploid. There is, moreover, no concentration in the trisomic class. The high frequency may have some explanation in that a

triploid, hybrid for one of 3 genomes (one genome of *P. integrifolia* and 2 of *P. axillaris*) might form more eggs that would contain more chromosomes homologous to those of *P. axillaris* and such eggs would function more frequently with haploid pollen of *P. axillaris* than with haploid pollen of *P. integrifolia*. It is most probable that the chromosomal complements of the various hybrid triploids are not identical and will produce a different distribution depending on the genetic make-up of the triploid. Although the number of plants involved in the particular 3n × 2n crosses is small, the percentages of distribution (table 6) seem to indicate that factors other than merely quantitative are operating to produce the distribution. The effects of

TABLE 6. *Distribution of progenies of individual 3n × 2n crosses in perigreed Petunia.*

	Groups					Cross
	I	II	III	IV	V	
Chromosome classes	14(2n)	15 16	17 18	19 20	21(3n)	
Number of plants	1	10 7	3 3	1 3	0	3n × 2n *P. integrifolia*
Group %	3.6	60.7	21.4	14.3	0.0	
Number of plants	3	2 2	1 2	2 5	0	3n × 2n *P. axillaris*
Group %	17.3	23.6	17.6	41.1	0.0	
Number of plants	0	4 6	2 0	0 0	0	3n × 2n *P. integrifolia*
Group %	0.0	83.4	16.6	0.0	0.0	
Number of plants	1	6 1	1 3	1 3	0	3n × 2n *P. integrifolia*
Group %	6.3	43.7	25.0	25.0	0.0	
Number of plants	0	1 1	1 2	1 4	0	3n × 2n *P. axillaris*
Group %	0.0	20.0	30.0	50.0	0.0	
Number of plants	3	1 1	0 0	1 1	0	3n × 2n *P. axillaris*
Group %	42.8	28.6	0.0	28.6	0.0	

specific factors for sterility and fertility are being analyzed by Dr. A. B. Stout. It may be noted that the inclusions of the various types of distribution of the individual crosses into a single general distribution produces a distribution that resembles that given by Levan (1942) for several genera but this masks the individual factors at work. The detailed analysis of each genotype in the cross is necessary if the true nature of such crosses is to be determined.

Triploid-Triploid Crosses. The reports of previous triploid crosses listed as the selfings of triploids, are found in Dermen (1931), Matsuda (1935), and Levan (1937). The triploid crosses reported on by the present author are different in that two presumably different hybrids are involved. The chromosome classes found in previous reports include the classes from the diploid 14 to 18 and from 26 to 29. It can be seen that the present report, giving the numbers 19, 20, 23, and 25, fills in the curve of distribution near

the triploid range that had been considered characteristically absent in triploid-triploid crosses in *Petunia* by Levan (1942). Levan compared the progenies of triploid-triploid crosses in 8 genera including *Petunia* and divided the range of the progenies into three groups comprising the ranges 2n to 3n – 1, 3n to 4n and 4n and upwards. Levan found that the progenies fell into two main groups, one with low basic haploid chromosome numbers and the other with high basic haploid numbers. The low basic haploid chromosome number group in which *Petunia* is found is subdivided according as the progeny of the triploids fell to the extent of 50 per cent or more into the lowest range, 2n to 3n – 1, or into the upper ranges to the same extent. The only member of the second group is *Populus* (Johnson 1942) which has a distribution that approaches the expected binomial distribution in the triploid progenies. Johnson (1942) and Levan (1942) suggest that this distribution is due to secondary polyploidy. The appearance in the author's material of chromosome classes in the triploid range would not argue against such a view but would stress that viability of the gametes is concerned with the formation of gametes with equal numbers of homologous chromosomes (Müntzing 1936). In this connection it may be noted that many authors have reported that the cross 2n × 3n invariably gives a high proportion of diploids and the progeny is limited to numbers close to the diploid. It would appear that equality of number in the gametes is an important factor in viability. Dermen (1931) reported that from such a cross there were reconstituted 39 diploids and 12 trisomics in 51 segregates, and Levan (1937) reported that the total progeny of such a cross consisted of 41 diploids. Apparently only haploid or disomic pollen functioned in these crosses. The work of Johnsn (1942) on *Populus* showed that the distribution of 2n × 3n and 3n ⤬ 2n was essentially the same, indicating that pollen grains are not more susceptible than embryo sacs to unbalanced chromosome numbers. There is every reason to believe that the pollen formed in triploids ranges from haploid to 2n – 1 from the observations of Dermen (1931) and from Satina and Blakeslee (1937a). It would seem that all classes of pollen function in the triploid crosses since nearly all classes between 2n and 4n are represented in the progenies. The numerical ratio of chromosomes in the egg and pollen grain is quite important as far as can be judged from the viable zygotes.

In the statistical analysis of triploid progenies of several genera, Levan (1942) found three types exemplified by *Beta* (Levan 1942), *Allium* (Levan 1936a, 1936b) and *Populus* (Johnson 1942). In *Beta*, the majority of the progeny fall between 2n and 3n and all theoretical expectations are well fulfilled. In *Allium*, however, most of the progeny approached the 4n class, while some of the lower classes are almost or entirely missing. In *Populus*, the entire range from 2n to 4n is well represented according to the

theoretical distribution expected, with maxima near 2n and 4n and a pronounced maximum at 3n. The analysis does not of course take into consideration the specific factors characteristic of each triploid used in the crosses. The very generally reported reduced fertility and frequent sterility of triploids and the reduced percentage of germination are probably dependent on the specific genotype of the individual triploid. Thus the counts reported for triploid-triploid crosses in *Petunia* indicate that there well may be a concentration at or near the 3n class depending on the specific genotypes employed in the cross.

The factors that influence the distribution of chromosome classes in triploid progenies away from the theoretically expected distribution are discussed at length by Levan (1942). The elimination of chromosomes appears to be a major factor in lowering the range of distribution. Upcott and Philip (1939) attribute much importance to the relative size of chromosomes and cells, in that small chromosomes will be more easily eliminated in large cells in which the distance to the poles from the equator is relatively long. Thus elimination would occur more easily in embryo sac mother-cells than in pollen mother-cells. Levan (1942) attributed little influence to these factors, nor did he find any significant relation between the functioning of pollen with the changed chromosomal relations of mother plant, embryo, and endosperm in polyploid crosses. Müntzing (1936) had suggested that such relations were of considerable importance.

In the analysis of triploid progenies of *Allium, Populus,* and *Beta,* Levan (1942) showed that less haploid pollen functions in zygotic production in 3n × 3n than in 2n × 3n crosses. Pollen grains with higher chromosome numbers are more successful in forming zygotes in the triploid than in the diploid crosses involving triploids. In the 3n × 3n crosses 2n pollen grains are much more frequent than in the cross 3n × 2n. One explanation is the need of numerical equality of chromosomes in egg and pollen (Müntzing 1936). Levan concludes that zygotic selection is the cause of differences in the progenies of triploids in the three genera mentioned above. Thus also there appears to be a positive selection for near-diploid pollen in the progenies of triploids in *Petunia,* since nearly three-fourths of all such progenies belong to the near-tetraploid class, yet from the evidence of Satina and Blakeslee (1937a) all classes of pollen seem to be formed in such triploids.

Triploid-Tetraploid Crosses. The previous reports on this type of cross in *Petunia* are found in Dermen (1931), Matsuda (1935), and Levan (1937). The present author lists only two segregates from such a cross. These, combined with previous reports, bring the total of such segregates reported to 54. Of this total, 45 fall in the 27–28 class (tetraploid range). It would seem here that diploid pollen is formed and functions mainly with

eggs of near- or exactly diploid pollen. The range of previous segregates from such crosses extended from the class 24 to 35 (pentaploid) with classes 30, 32, 33, and 34 missing. Thus the plant with 20 chromosomes reported by the author as a segregate from the triploid-tetraploid cross is the lowest count for such a cross in *Petunia*. However, extreme variation in the progenies of similar crosses in other genera are reported. Müntzing (1937) reported in the genus *Dactylis* that the progeny included the classes from 3n + 1 to 8n. Müntzing noted the maximum present in the tetraploid range from such crosses. It may be suggested that the 20-chromosome plant reported by the present author originated from gametes with approximately equal numbers such as 10 and 10 or 11 and 9. The possibility of homology existing between such gametes seems relatively great in a cross between an autotetraploid and a triploid containing 2 genomes that are supposedly homologous with the genomes of the tetraploid.

The Selfing of Hexaploids. This is the first report of hexaploids in *Petunia*. The hexaploids were produced by colchicine treatment of a branch of a hybrid triploid (field no. 170–4) which contained 2 genomes of *P. axillaris* and 1 genome of *P. integrifolia*. These genomes are presumably doubled in the hexaploid. Twenty-five plants of the selfings of the colchicine-treated branch were examined. Exact hexaploids (42) were found in 16 plants, 6 plants had 40 chromosomes, and 3 plants were in the 41 class. Of the 50 plants examined from the selfings of derived hexaploids 26 were exact hexaploids, 10 were in the 40-chromosome class, 6 in the 41 class, 7 in the 43 class and 1 in the 44 class. It is evident that the colchicine-induced hexaploids and their derived progeny possess a mechanism for fairly stable sexual reproduction. The allopolploidy present in the hexaploids does not seem to disturb the stability. Müntzing (1936) considers that most natural polyploid races which flourish widely are allopolyploid to some degree. The attempt to identify specific chromosomes was unsuccessful, and since no meiotic studies have yet been made on these hexaploids, it is impossible at present to assign any cause for the stability of the hexaploids. However, it should be mentioned that the factors governing incompatibility in the diploid *P. axillaris* were inactivated in the change from the diploid to the tetraploid condition (Stout & Chandler 1941, 1942; Stout 1945). The stability of the sexual reproduction of the tetraploid is reflected in the hexaploid. It may then be that homology and numerical equality of chromosomes in egg and pollen are the main factors in this stability.

Hexaploid-Tetraploid Crosses. The distributions of chromosome classes in the progenies of hexaploid-tetraploid crosses are different according as a different hybrid hexaploid is used as the seed parent (table 4). It is of

course extremely improbable that the chromosome complements of any two hybrid hexaploids are identical. However, although the maximum in the distribution (table 4) in one cross is the chromosome class 33 and in the other it is the class 35 (pentaploid), there is still a pronounced concentration in the latter in the class 33. The differences in genotype alone do not seem to effect wide differences in the distributions in the progeny and in both crosses these same classes include most of the progeny. In the latter cross the entire distribution is shifted to the higher classes and includes two hyperpentaploids. In general it may be said that the eggs are formed with numbers close to the triploid and the pollen is formed with numbers close to the diploid. This might be expected in view of the stability of both tetraploids and hexaploids.

The sole plant from the reciprocal cross, 4n × 6n, was found to be an exact pentaploid. This is in agreement with the general observation that pollen grains with euploid numbers have a greater viability (Levan 1937). While the pollen may have been exactly triploid and the egg exactly diploid, slightly aneuploid gametes would probably have given the same result.

The high rate of constancy in the progenies of both selfed hexaploids and of the hexaploid-tetraploid crosses is perhaps due to the presence of 4 genomes of *P. axillaris* in both the hexaploid and tetraploid. Homology exists to a large extent between the tetraploid and the hexaploid and in this respect the hexaploid shows the characteristics of an autopolyploid. Müntzing and Müntzing (1942) in crosses between diploid and three different hexaploid biotypes of *Potentilla* obtained 28 pentaploids. These authors ascribe the pentaploid formation to the unreduced ovules of the diploid and the reduced (triploid) pollen of the hexaploids. These authors concluded that the result of a cross in *Potentilla* material is determined not only by the degree of sexuality present (apomixis may be present) but by the chromosome number and the genetic constitution of the male parent. Nordenskiöld (1937) found in *Phleum* that a cross of a triploid with a hexaploid gave mostly hexaploid plants. He ascribed this to the viability of the unreduced triploid gametes. In a hexaploid-tetraploid cross he obtained only three plants but all were pentaploids. He concluded that in the crosses in *Phleum* the four types used were closely related and the genomes more or less homologous. The diffi·ulties encountered in crossing the various types were ascribed to quantitative differences in chromosome numbers rather than to any qualitative differences. It may also be said of the hexaploid crosses in *Petunia* that the viable zygotes are usually formed from eggs and pollen with approximately the same number of chromosomes. This number is euploid or only very slightly aneuploid. However, the loss or gain of one or two extra chromosomes does not affect the functioning of eggs and pollen in the higher polyploid forms. One hyperhexaploid (41) gave on selfing 11 plants. All the plants fell within

the chromosome classes 40 to 44 and 63.6 per cent were in the balanced hexaploid class (42). A similar range was found in the progeny of the balanced hexaploids.

SUMMARY

1. The leaf smear technique combined with various pretreatments has been used to determine the somatic chromosome numbers of a pedigreed population of *Petunia* of Dr. A. B. Stout.

2. Confirmations of the diploid number of 14 for the pure species of *P. axillaris, P. integrifolia,* and *P. parodii* were made.

3. The chromosomes of the three diploid species are described as being within the range of 2.5 to 4.5 μ in length by 0.6 μ in width at metaphase. A SAT-chromosome is present in *P. axillaris*.

4. A chromosome fragment was reported in a pure diploid *P. parodii* and in a segregate from a hybrid 3n × 2n cross.

5. Confirmatory determinations of tetraploid forms of *P. axillaris* were made.

6. The progeny of 4n *P. axillaris* × 2n *P. integrifolia* consisted of 6 triploid and one hypotriploid with 20 chromosomes.

7. Fourteen plants from the cross 4n *P. axillaris* × 2n *P. axillaris* were determined as exact triploids and one as diploid (14).

8. All the chromosome classes between 2n and 3n − 1 were found in both the crosses hybrid 3n × 2n *P. integrifolia* and hybrid 3n × 2n *P. axillaris*. The distribution of the chromosome classes in the progeny of the latter cross tended to fall among the upper classes in the range 2n to 3n − 1. Comparison is made with similar previously reported crosses in *Petunia*.

9. The progeny of hybrid triploid-triploid crosses included 13 plants in the range of hypotriploid (19) to tetraploid (28). The chromosome classes 19, 20, and 23 are reported for the first time for such progenies.

10. Hybrid 3n × 4n *P. axillaris* gave only two plants, one with a count of 20 chromosomes and the other with 26 chromosomes.

11. Seventy-five plants, the progeny of the selfing of a colchicine-induced hexaploid hybrid and derived hexaploids, were all found to have chromosome numbers within the range from 40 to 44. Fifty-six per cent of the progeny were exact hexaploids.

12. The progenies of crosses of hybrid hexaploids × 4n *P. axilaris* all fell within the chromosome classes from 29 to 36 but the class 30 was not represented. Twenty-five per cent of the progenies were exact pentaploids. Individual crosses showed minor differences in distribution due perhaps to the different chromosome complements of the seed parent.

13. The reciprocal cross 4n *P. axillaris* × hybrid hexaploid gave only one plant with a count of 35 (pentaploid).

14. A pretreatment using podophyllin and chloral hydrate either in sequence or in combination was reported as a satisfactory substitute for colchicine for the study of the morphology of chromosomes.

15. A comparison with previous reports of similar crosses in *Petunia* was made with a discussion concerning the possible factors operating to produce chromosomal variation in the progenies.

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A SURVEY OF 500 BASIDIOMYCETES FOR ANTIBACTERIAL ACTIVITY

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A previous publication from this laboratory (7) presented the results of a survey for antibacterial activity of more than 400 fungi, including over 300 Basidiomycetes, against *Staphylococcus aureus* and *Escherichia coli*. The present paper is a report of a survey of the antibacterial activity of 508 additional isolations of Basidiomycetes against the same indicator organisms. The procedures used were substantially those of the earlier investigation; differences are noted later in this paper.

Recent studies of the Basidiomycetes suggest that they are a promising source of antibiotic substances. Wilkins and Harris (12) tested extracts of the sporophores of 722 species in 96 genera of the larger Basidiomycetes for antibacterial activity against *Staph. aureus, E. coli,* and *"Pseudomonas pyocyanea."* They used the "hole" method (10) and a fungus was given a positive rating if the zone of inhibition of the bacterium was at least 2 mm. About 20 per cent were positive against *Staph. aureus* alone or *Staph. aureus* and *E. coli.* None was active on *E. coli* alone. Five species were active with *"P. pyocyanea."*

Wilkins (8) tested 56 isolations of Basidiomycetes grown on liquid and agar media. The liquids were tested by the "hole" method and the cultures grown on agar were tested by the "strip" method (11). Liquids active against *Staph. aureus* were obtained with 28 of the cultures; 11 were active against *Staph. aureus* and *E. coli;* 3 were active against *E. coli* and not against *Staph. aureus.*

Wilkins (9) reported the results on 100 additional Basidiomycetes grown on agar and in liquid, and tested by the "strip" and "hole" methods respectively against *Staph. aureus* and *E. coli.* About 10 per cent were strongly positive, 20 per cent were weakly positive and the rest were negative.

Robbins and his associates (7) in a survey of over 400 fungi, including over 300 Basidiomycetes, found that about 50 per cent displayed antibacterial activity. One hundred nineteen fungi produced zones of inhibition of more than 10 mm. for *Staph. aureus* by the streak method. None was active against *E. coli* alone. Two fungi were found to produce culture fluids active against *Staph. aureus* at a dilution of 1 to 1024.

Hollande (4) reported that clitocybine, an antibiotic substance from *Clitocybe gigantea* var. *candida* is active against several bacteria including *Mycobacterium tuberculosis, Bacillus typhosus,* and *Brucella abortus.* Posi-

tive therapeutic results were reported on guinea pigs infected with tuberculosis.

Mathieson (6) tested the extracts of the sporophores of 230 species of Australian Basidiomycetes for antibacterial activity. He used the "hole" method of Wilkins and Harris. An inhibition zone of 2 mm. was considered positive. One hundred seventy gave negative results; 39 were active against *Staph. aureus* alone; 20 were active against *Staph. aureus* and *E. coli;* and one was active against *E. coli* alone.

In an investigation of over 200 types of toadstools and mushrooms, Atkinson (1) found only five with activity against *Staph. aureus.* Two organisms, *Cortinarius rotundisporus* and *Psalliota xanthoderma,* were active against *Staph. aureus* and *"B. typhosum."* Further work on extracts of the sporophores from these two species indicated that both fungi were active against a wide range of bacteria. The activity of both extracts was unaffected by the presence of 10 per cent serum, and the toxicity to animals was low.

Bose (2) reported that polyporin, the filtrate of *Polystictus sanguineus* grown in a supplemented Czapek-Dox medium, is active against *Staph. aureus, Streptococcus pyogenes, Streptococcus viridans, B. typhosus, B. paratyphosus* A and B, *E. coli, Vibrio cholerae,* and *B. flexner.* Polyporin was stated to be non-toxic to animals.

METHODS AND MATERIALS

Five hundred and eight isolations of Basidiomycetes were tested for antibacterial activity against *Staph. aureus* and *E. coli.* The streak method was used as a preliminary screening test. The fungi found to be most effective in inhibiting the growth of one or both indicator bacteria and which were not strong acid producers were tested further for activity by the agar disc method. The fungi displaying marked antibacterial activity by both the streak and disc methods were then cultivated in liquid media and the antibacterial activity of the culture fluids was determined by serial dilution tests. Several related problems were investigated as described later.

The fungi were obtained from various sources.[1] Five isolations came from the Centraalbureau voor Schimmelcultures, Baarn, Holland (CBS), 49 from Dr. Dow V. Baxter, University of Michigan (B), 159 from Dr. José Emilio Santos Pinto Lopes, Instituto Botanico, Lisbon, Portugal (L), and 295 from Dr. Mildred K. Nobles, Central Experimental Farm, Ottawa, Canada (N).[2] These cultures included 64 genera and 352 species. Twelve isolations were unidentified as to species.

    [2] The letters in parentheses are used in this report to indicate the source of the cultures. Hereafter, these letters will be used before the stock numbers of the cultures.

The assistance of Dr. Baxter, Dr. Lopes, and Dr. Nobles in supplying the fungi tested in this survey is appreciated. It would not have been possible to have completed the work reported here without the cultures so freely and generously supplied.

The author wishes to thank Dr. G. W. Rake, Squibb Institute for Medical Research, for the, cultures of *Staphylococcus aureus* (Heatley strain) and *Mycobacterium phlei*, Dr. Selman A. Waksman, New Jersey Agricultural Experiment Station, for the culture of *Escherichia coli*, and Dr. H. Boyd Woodruff, Merck and Co., Inc., for the culture of *Pseudomonas aeruginosa*.

Details on the methods and media are included in the sections which follow.

EXPERIMENTAL WORK

Survey by the Streak Method.   The isolations of fungi used in this survey were first tested for antibacterial activity by the streak method. Two media were found to be generally suitable for the growth of the fungi and of the indicator bacteria. These were a modified Czapek-Dox medium prepared with 5 g. corn-steep solids per liter[3] and a thiamine peptone agar.[4] Each fungus was grown on 20 ml. of each medium in Petri dishes 15 × 100 mm. at 25° C. After several days, depending upon the rapidity of growth of the fungus, the plates were streaked with 6–8 hour AC[5] broth cultures of *Staph. aureus* and *E. coli* starting at the edge of the fungus colony and extending to the periphery of the dish. The plates were incubated overnight at 37 ± 2° C and when inhibition of the indicator bacteria was observed measurements were taken in millimeters from the edge of the fungus colony to the growth of the bacteria. Alkacid indicator paper (Fisher Scientific Co.) was used to test the acidity next to the fungus colony. Each isolation was usually tested for antibacterial activity at two different ages for each medium. The age of the colony, when tested, ranged from 4 to 28 days with two exceptions. The second tests on *Polyporus amorphus* (N16597) and *Omphalia campanella* (N11761) were made after 38 days because of the very slow growth of these organisms on the media used.

Results with the Streak Method.   Two hundred and thirty-three fungi showed no activity against *Staph. aureus* or *E. coli* on both media. These were :[6]

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[3] The solution contained per liter of distilled water 3 g. NaNO₃, 1 g. KH₂PO₄, 0.5 g. KCl, 0.5 g. MgSO₄ · 7H₂O, 40 g. dextrose and 15 g. Difco agar. The corn-steep solids were in Special Nutrient #14 from A. E. Staley Co. The pH was 5.7 after autoclaving.

[4] The thiamine peptone agar contained per liter 1.5 g. KH₂PO₄, 0.5 g. MgSO₄ · 7H₂O, 1 g. neopeptone, 10 g. dextrose, 15 g. Difco agar and 600 mμmoles of thiamine. Mineral supplements in p.p.m. were added as follows: 0.005 B, 0.02 Cu, 0.10 Fe, 0.01 Ga, 0.01 Mn, 0.01 Mo and 0.09 Zn. The pH was 5.5 after autoclaving.

[5] The AC broth contained per liter 20 g. proteose peptone #3, 3 g. yeast extract, 3 g. meat extract, 3 g. malt extract, 5 g. dextrose. The pH was 6.8 after autoclaving.

[6] An asterisk signifies that a disc test was made.

Agrocybe semiorbicularis (L92)
Armillaria mellea (L255)
Auricularia auricula (N16631)
Clitocybe geotropa (L179)
C. illudens (N10018)
C. tabescens (L210, NF2337)
Collybia fusipes (L204)
C. radicata (NF3461)
C. velutipes (NF1598)
Conocybe pubescens (L57)
C. lateritia (L464)
Coprinus boudierii (L60, L60.6, L268)
C. coopertus (L218)
C. curtus (L1, L141, L380)
C. domesticus (L2, L166, L379)
C. ephemerus (L121)
C. fimetarius (L414)
C. fimetarius (?) (L181)
C. friesii (L142)
C. hiascens (L200, L285, L378)
C. impatiens (L462)
C. lagopus (L4)
C. micaceus (NF290, L350, L364)
C. radians (L17, L86, L87)
C. radiatus (L374)
C. stercorarius (L91)
C. sp. (L375, L397, L397.4, L397.5)
Corticium alutaceum (NF6328)
C. coeruleum (NF2936)
C. galactinum (N16568)
Crucibulum vulgare (L438)
Cyathus striatus (L437)
Daedalea ambigua (NF 2017)
D. biennis (NF7302)
D. confragosa (N9210)
D. gibbosa (NF7309)
Deconica crobula (L107)
D. inquilina (L54.27)
Drosophila appendiculata (L25.8, L108)
D. badia (L242, L242.14, L341)
D. candolleana (L202)
D. exalbicans (L127, L127.4)
D. fatua (CBS22)
D. gracilis (L460)
D. lactea (L459)
D. pygmaea (CBS24, L391.6)
D. spadicophylla (L448)
D. sp. (L376, L444)
Favolus canadensis (N11794)
Flammula carbonaria (L66)
F. gummosa (L161)
F. ochroleuca (L315)
Fomes calkinsii (NF1819)
F. conchatus (NF10159)
F. connatus (N10254)
F. everhartii (NF3580)

F. extensus (NF7325)
F. fomentarius (N11264)
F. fraxineus (NF7519)
F. geotropus (NF2023)
F. igniarius (N8207)
F. igniarius laevigatus (NF1956)
F. lobatus (NF8008)
F. marmoratus (NF2024)
F. nigricans (B707)
F. nigrolimitatus (N10085A)
F. noxius (N9229)
F. rimosus (NF2164)
F. robustus (NF1656)
F. robustus tsuginus (N8219)
F. scutellatus (NF3444)
Fomitiporia dryophila (B713, B714)
F. earleae (B715)
F. flavomarginata (B716)
Galera tibicystis (L94)
Grandinia granulosa (N16567)
Lentinus lepideus (N11770)
L. tigrinus (L38.1, L38.3, L38.11)
L. tigrinus (?) (NF2030)
Lenzites betulina (N10199)
L. flaccida (NF7304)
L. repanda (N9231)
L. thermophila (NF1682)
L. tricolor (NF7327)
Lycoperdon gemmatum (NF2944)
Melanoleuca cognata (L63)
Merulius corium (L321)
Mucidula radicata (L111)
*_Mycena atroalba_ (L359)
Panus rudis (NF1927)
Peniophora corticalis (L360)
P. cremea (NF2946)
P. gigantea (NF7329)
P. populnea (N10740)
Phlebia mellea (N16566, N16569, N16626, N16642)
Pholiota adiposa (N8457)
P. aurivella (N8485)
P. mutabilis (L208)
P. spectabilis (NF6627)
P. squarrosa (L313)
Pholiotina togularis (L465)
Pleurotus ostreatus (N9220, L361)
P. sapidus (NF1610)
P. serotinus (NF2174)
Polyporus abietinus (N9517)
P. adustus (N11810)
*_P. alboluteus_ (N8263, N16598)
P. albosordescens (NF7330)
P. anceps (NF3372, N16565, N16565K)
P. arcularius (NF3768)
P. benzoinus (NF6606, B725)

P. borealis (N16610)
P. brumalis (N11804)
P. cinnabarinus (NF1609)
P. circinatus (N10910)
P. circinatus dualis (N11617)
P. conchifer (N10210)
P. croceus (NF2178)
P. cuticularis (N10194)
P. distortus (NF8002)
P. dryadeus (N9238)
P. dryophilus (NF2179)
P. elegans (N11785)
P. fibrillosus (N10909)
P. frondosus (N10235)
P. fumosus (N11813)
*P. galactinus (NF3494)
P. glomeratus (N11254)
P. graveolens (NF2954)
P. hirsutus (N11805)
P. hispidus (NF2038)
P. imberbis (N9232)
P. lucidus (N10222)
P. pargamenus (NF8016)
P. picipes (NF1352)
P. pubescens (N11616, N16660)
P. resinosus (N11611*, N16625, N16659)
P. robiniophilus (NF2185)
P. spumeus mongolicus (NF2288)
P. squamosus (N10789)
P. tamaricis (NF7337)
P. tenuis (NF2048)
P. tuberaster (NF7997b)
*P. tulipiferus (NF8049)
P. umbellatus (N9263)
P. velutinus (N10238)
P. versicolor (N11812)
P. zonatus (NF7389)
Poria albipellucida (N11692, B737)
P. albobrunnea (B738)

P. ambigua (N8448)
P. aurea (N16609)
P. cinerascens (N9267)
P. crustulina (N16600)
P. ferruginosa (N11253)
P. floridana (B767)
P. hymenocystis (NF7315)
P. laevigata (B770, B771)
P. mollusca (B777)
P. obliqua (NF7403)
P. prunicola (NF1409)
P. pulchella (B784)
P. punctata (NF1563)
P. rufa (N16560, N16564, N16615, N16616, B786)
P. semitincta (B787)
P. sericeo-mollis (NF8017)
P. subfusco-flavida (B795)
P. tacamahacae (B796)
P. tsugina (B797)
P. undata (B799)
P. xantha crassa (B808)
P. sp. (N16646, B789)
Pseudocoprinus disseminatus (L16)
Radulum casearium (N16599)
Schizophyllum commune (L443, N11795)
Stereum abietinum (N16603)
S. fasciatum (NF7341)
S. gausapatum (N9246)
S. rugosum (N9245)
S. sanguinolentum (N9504)
S. spadiceum (N8644)
Stropharia merdaris (L23, L223)
Trametes gibbosa (L319)
T. hispida (NF6005)
T. robiniophila (NF3773)
*T. serpens (B)
T. suaveolens (NF7654)
Tricholoma ambustum (L269)

Zones of inhibition less than 5 mm. against *Staph. aureus* were obtained with the following 26 isolations:

Armillaria mellea (NF2508)
Clitocybe phyllophila (L463)
Clitopilus abortivus (NF3517B6)
Coprinus extinctorius (L203)
C. hiascens (L200.3)
Drosophila appendiculata (L20, L84)
D. caudata (L458)
D. marcescibilis (CB826)
D. prona (L332)
Fomes fulvus (NF2283)
*Lenzites saepiaria (N7528)
Panaeolus retirugis (L18)

Peniophora quercina (NF8089)
*Pluteus nanus (L225)
Poria eupora (N11252, B753)
P. mutans tenuis (B779)
P. viridiscula (B801)
*P. weirii (N9422)
*P. xantha (N9315)
P. xantha f. crassa (B811)
Stereum abietinum (N16604)
S. sulcatum (N16619)
Trametes mollis (NF2351)
T. tenuis (N9265)

Most of these fungi showed no inhibition of *E. coli*. *Armillaria mellea* (NF2508), *Clitocybe phyllophila* (L463), *Lenzites saepiaria* (N7528), and *Peniophora quercina* (NF8089) produced zones of inhibition less than 5 mm. on *E. coli*. Zones of inhibition slightly more than 5 mm. were produced with *Pluteus nanus* (L225) and *Stereum sulcatum* (N16619).

Zones of inhibition for *Staph. aureus* ranging from 5 to 10 mm. as a maximum were produced by 46 of the isolations tested. There were:

Corticium effuscatum (NF5322)	*P. betulinus* (N16656)
Daedalea flavida (NF7301)	*P. borealis* (NF3581)
Drosophila candolleana (L202.1, L202.5)	*P. caesius* (NF6891)
D. sarcocephala (L96)	*P. gilvus* (NF1965)
D. sp. (L382)	**P. hydnoides* (NF2039)
Fomes ellisianus (NF7520)	**P. oregonensis* (N8240)
F. igniarius robustus (N9228)	**P. sulphureus* (NF3474)
F. nigrolimitatus (B708)	**P. tsugae* (N9346)
*F. pini (N16632)	*Poria alaskana* (B736)
F. subroscus (NF1639)	*P. crustulina* (B751)
*Hydnum abietis (N16602)	*P. cupora* (B752)
*Hymenochaete tabacina (N9252)	**P. ferrea* (N9887)
Lentinus kauffmanii (N11660)	*P. medullapanis* (B775)
*Lenzites trabea (NF3823)	*P. nigrescens* (N16605)
Merulius confluens (NF2945)	*P. rufa* (N16617)
*Panaeolina foenisecii (L10)	*P. weirii* (N16607)
Panaeolus campanulatus (L466)	*P.* sp. (B790)
P. papilionaceus (L387.8, L468)	**Stereum pini* (NF2963)
Pholiota destruens (NF1566)	**S. subpileatum* (NF2056)
P. mutabilis (L208.5)	**Trametes isabellina* (NF7338)
P. sphaleromorpha (L132)	**T. serpens* (B832)
Polyporus berkeleyi (NF2952)	*T. variiformis* (N16572)

The antibacterial activity of most of these fungi as indicated by the streak test was limited to *Staph. aureus*. With *Fomes ellisianus* (NF7520). *Lenzites trabea* (NF3823), *Pholiota sphaleromorpha* (L132), *Stereum pini* (NF2963), and *S. subpileatum* (NF2056) there was an inhibition zone of less than 5 mm. on *E coli*. The zone of inhibition for *E coli* ranged from 5–10 mm. as a maximum with *Fomes pini* (N16632), *Hymenochaete tabacina* (N9252), *Panaeolina foenisecii* (L10), *Polyporus berkeleyi* (NF2952), *P. betulinus* (N16656). *Poria ferrea* (N9887), *Trametes isabellina* (NF7338), and *T. variiformis* (N16572). One fungus in this group, *Polyporus caesius* (NF6891), produced a larger inhibition zone with *E. coli* than with *Staph. aureus*.

Inhibition zones of more than 10 mm. for *Staph. aureus* were obtained with 203 of the 508 isolations on one or both media used in the survey. The results for these fungi are summarized in table 1. For each of these fungi the largest zones of inhibition against *Staph. aureus* and *E. coli* on both the corn-steep and the thiamine peptone agars are given. The A in the last column designates that the fungus produced a considerable amount of acid.

TABLE 1. *Antibacterial activity of fungi tested by streak method.* The maximum inhibition zones in mm. are given against *Staph. aureus* and *E. coli* for the fungi grown on the corn-steep or the thiamine peptone agar. "A" indicates that a considerable amount of acid was produced by the fungus. Those marked with an asterisk were also tested by the agar disc method.

Organism	Corn steep agar		Thiamine peptone agar		Acidity
	Staph. aureus	*E. coli*	*Staph. aureus*	*E. coli*	
Agrocybe dura (L386)	30	30	60	51	
A. dura (L386.10)	50	50	50	50	
A. dura (L386.14)	50	50	55	55	
A. semiorbicularis (L92.1)	16	10	20	22	
A. semiorbicularis (L92.6)	23	11	30	9	
Alnicola escharoides (L205)	35	0	18	0	
A. melinoides (L206)	10	0	15	0	
Claudopus nidulans (NF1996)	21	3	22	8	
C. nidulans (N16627)	25	4	45	8	
Clitocybe ditopa (L357)	20	12	30	17	
C. obsoleta (L358)	70	13	·75	3	
C. robusta (NF2932)	16	0	35	0	
Collybia dryophila (L62)	16	14	40	40	A
C. dryophila (L72)	29	29	45	38	A
C. dryophila (L109)	18	18	29	29	
C. erythropus (L30)	31	25	40	32	
Coniophora puteana (N9779)	11	5	6	6	
C. sp. (N16648)	3	4	14	16	
Coprinus auricomus (L58)	20	0	23	0	
C. comatus (NF2935)	2	2	25	23	
C. picaceus (L14)	32	0	30	0	
C. plicatilis (L461)	25	0	40	0	
C. similis (L3)	8	8	15	11	
Corticium portentosum (N9283)	30	0	4	0	
Crepidotus mollis (L73)	0	0	15	20	
Daedalea quercina (NF2278)	10	10	23	18	A
D. unicolor (N10202)	22	0	7	0	
Deconica inquilina (L54a)	20	0	0	0	
D. inquilina (L54b)	17	0	10	0	
Drosophila appendiculata (L25)	45	0	30	0	
D. caudata (L277)	30	0	8	0	
D. caudata (L277.7)	27	0	37	0	
D. caudata (L278)	70	0	60	0	
D. exalbicans (L450)	59	0	65	30	
D. fibrillosa (CBS23)	70	0	50	0	
D. fibrillosa (L15)	42	0	60	0	
D. fibrillosa (L152)	52	0	57	0	
D. gracilis (L171.4)	35	0	35	0	
D. hydrophila (L474)	0	0	25	0	
D. orbitarum (L445)	5	0	12	0	
D. semivestita (L281)	50	25	62	33	
D. subatrata (CBS25)	51	24	60	42	
D. subatrata (L43.1)	60	42	69	27	
D. subatrata (L122)	55	24	50	50	
D. subatrata (L284)	53	29	63	30	
D. subatrata (L284.3)	56	34	65	35	
D. subatrata (L284.6)	60	20	71	31	
D. subatrata (L449)	46	9	60	31	
D. trepida (L247)	33	0	24	0	

TABLE 1 (*Continued*)

Organism	Corn steep agar		Thiamine peptone agar		Acidity
	Staph. aureus	*E. coli*	*Staph. aureus*	*E. coli*	
D. trepida (L247.3)	60	0	29	7	
D. vernalis (L367)	48	0	60	30	
D. sp. (L395.2)	27	0	0	0	
Echinodontium tinctorium (N8740)	37	0	22	0	
Flammula sapinea (L31)	16	0	21	0	
Fomes annosus (N8273)	33	17	55	21	
F. applanatus (N9295)	0	0	17	15	
F. fraxinophilus (NF1931)	0	0	19	18	
F. juniperinus (NF3765)	7	5	48	46	
F. meliae (NF2025)	27	12	30	23	A
F. nigrolimitatus (N16651)	0	0	12	6	
F. nigrolimitatus (N16652)	0	0	20	13	
F. officinalis (N9503)	65	1	55	30	A
F. pini (NF2162)	19	18	25	21	A
F. pini (N16612)	60	41	48	42	A
F. pini (N16635)	18	18	18	15	A
F. pinicola (N8568A)	15	12	18	17	A
F. ribis (NF2284)	14	10	10	7	
F. roseus (NF5565)	15	10	18	13	
F. spadiceus (N8102)	0	0	30	0	
Hydnum abietinum (N16601)	3	0	17	0	
Hypholoma sublateritium (NF2168)	6	0	11	0	
Hypochnus centrifugus (NF6896)	17	12	25	17	A
Irpex cinnamomeus (NF2169)	40	40	35	20	A
I. mollis (NF3047)	6	0	15	0	
Lentinus cochleatus (L130)	15	0	22	0	
L. degener (L45)	5	5	24	20	
L. tigrinus (L38.2)	55	0	25	35	
Lenzites bicolor (NF7326)	11	4	10	0	
Lepiota naucina (NF2170)	24	20	25	15	
Marasmius candidus (L149)	7	0	19	0	
Merulius corium (L273)	12	12	0	0	
M. lachrymans (NF3526)	35	28	65	55	A
Mycena capillaris (L291)	20	0	21	0	
Omphalia campanella (N11761)	0	0	38	0	
O. flavida (NF5711)	15	15	9	8	
Panaeolus acuminatus (L467)	12	7	5	0	
P. campanulatus (L371)	0	0	15	0	
P. papilionaceus (L387.2)	15	0	0	0	
P. papilionaceus (L387.3)	55	3	30	0	
P. papilionaceus (L387.9)	60	15	50	6	
P. sub balteatus (L330)	55	0	15	0	
P. sub balteatus (L482)	15	0	2	0	
Panellus stypticus (L369)	15	16	39	26	A
Paxillus panuoides (NF3007)	22	12	27	8	
Peniophora incarnata (NF8090)	70	70	26	5	
P. pubera (NF3456)	0	0	12	0	
Pholiota lucifera (NF673)	15	4	24	13	
P. sphaleromorpha (L132.2)	11	8	28	28	
P. sphaleromorpha (L132.3)	11	10	4	7	
P. sphaleromorpha (L132.6)	11	10	15	14	
Pleurotus griseus (NF2948)	23	0	13	0	
P. ulmarius (NF2950)	55	0	25	0	

TABLE 1 (*Continued*)

Organism	Corn steep agar		Thiamine peptone agar		Acidity
	Staph. aureus	E. coli	Staph. aureus	E. coli	
Polyporus abietinus (N16634)	0	0	11	5	
*P. albellus (N11608)	18	0	22	0	
*P. amorphus (N8552)	42	7	32	10	
*P. amorphus (N16597)	68	0	45	0	
*P. amorphus (N16613)	38	0	35	7	
*P. balsameus (N8407)	0	0	12	9	
*P. betulinus (N11610)	0	0	20	18	
*P. caesius (NF6891)	7	0	10	13	
*P. compactus (NF8004)	10	0	14	0	
*P. dichrous (N11609)	9	5	18	17	
*P. dryophilus vulpinus (NF4394)	0	0	30	26	A
P. durus (N9234)	6	6	20	20	A
*P. fragilis (N10227)	12	3	18	3	
*P. giganteus (NF3769)	31	31	6	0	A
*P. guttulatus (N11603)	0	0	22	15	
P. helveolus (NF7334)	10	7	26	25	A
*P. immitis (?) (N10215)	37	37	57	47	A
P. intybaceus (NF7311)	0	0	15	14	
*P. mollis (NF6827)	21	15	45	45	
*P. montanus (N8449)	0	0	15	5	
*P. nidulans (N9312)	0	0	25	0	
*P. obtusus (NF6798)	20	0	25	0	
*P. osseus (N11701)	0	0	25	16	
P. palustris (N10618)	12	11	22	16	A
*P. radiatus (N10229)	17	0	24	0	
*P. schweinitzii (NF7448)	5	4	25	0	
*P. semipileatus (NF3499)	40	9	30	12	
*P. spraguei (NF2182)	0	0	16	12	
P. stypticus (N9223)	41	39	59	51	A
*P. subcartilagineus (N9417)	11	13	49	50	A
*P. tephroleucus (?) (N10902)	30	20	21	18	
P. vaporarius (NF1287)	20	14	35	35	A
P. vaporarius (B735)	20	20	35	35	A
P. vulpinus (NF2279)	20	18	8	0	A
*P. zonalis (NF2049)	41	0	5	0	
*Poria albobrunnea (N16574)	14	9	10	10	
*P. candidissima (N11613)	5	0	30	0	
*P. carbonica (N8247)	0	0	25	23	
*P. colorea (N11791)	26	0	8	0	
*P. ferrugineo-fusca (NF1411)	12	0	27	5	
P. ferrugineo-fusca (N16614)	5	0	21	8	
*P. ferrugineo-fusca (N16621)	17	0	31	0	
*P. hypolateritia (N9249)	11	0	7	0	
P. incrassata (NF7316)	2	0	35	27	A
*P. lenis (B772)	23	21	31	29	A
*P. microspora (N10724)	0	0	25	22	A
P. pearsonii (N9224)	6	0	60	60	A
*P. sequoiae (N8755)	2	0	13	10	
*P. sitchensis (B788)	2	0	39	16	
P. spissa (B791)	12	13	38	34	A
*P. subacida (N9762)	32	0	22	8	
*P. subacida (N16636)	26	0	7	0	
*P. unita (N11259)	8	0	54	55	A

TABLE 1 (*Continued*)

Organism	Corn steep agar		Thiamine peptone agar		Acidity
	Staph. aureus	E. coli	Staph. aureus	E. coli	
*P. vaillantii (N10444)	5	0	14	8	
*P. versipora (B800)	28	0	14	0	
*P. vulgaris (B802)	0	0	12	0	
*P. weirii (N16606)	16	26	17	35	
*P. xantha (N16570)	18	14	40	38	A
*P. xantha (N16571)	13	11	35	34	A
*P. xantha (N16629)	0	0	30	23	A
P. xantha (N16630)	0	0	29	25	A
P. xantha (N16645)	5	0	35	35	A
*P. xantha (N16647)	12	15	27	25	A
*P. xantha crassa (B805)	17	20	35	35	A
*P. xantha crassa (B806)	20	21	25	24	A
*P. xantha crassa (B807)	12	6	46	40	A
*P. xantha f. crassa (B809)	11	10	40	38	A
*P. xantha f. crassa (B810)	0	0	21	16	A
*Rhizoctonia crocorum (NF6812)	14	0	0	0	
*Rhodopaxillus nudus (L352)	53	24	49	0	
*Schizophyllum commune (N16654)	0	0	15	10	
*Sparassis crispa (N9244)	0	0	18	10	
*Stereum abietinum (N11792)	13	0	20	10	
*S. frustulosum (NF2055)	0	0	24	10	
*S. hirsutum (N9282)	16	10	18	11	
*S. murrayi (N11362)	14	13	17	13	
*S. purpureum (NF682)	0	0	16	0	
*S. sanguinolentum (N16618)	21	20	12	7	
*S. sulcatum (N8116)	42	30	28	22	
*S. sulcatum (N16563)	28	17	49	43	
*Stropharia mammillata (L230)	20	0	13	0	
*S. merdaria (L223.15)	0	0	14	12	
*Trametes americana (N9414)	24	19	18	0	
*T. carbonaria (N8432)	11	8	11	10	
T. feei (NF7320)	7	7	41	42	A
*T. heteromorpha (N8440)	24	0	42	37	A
*T. heteromorpha (N16558)	46	4	50	50	A
*T. heteromorpha (N16620)	30	9	32	35	A
*T. isabellina (B820)	0	0	24	18	
*T. isabellina (B821)	0	0	20	20	
T. lilacino-gilva (NF7322)	0	0	22	20	A
T. morganii (B822)	0	0	15	14	
*T. serialis (N10915)	0	0	27	30	A
*T. serialis (N16561)	15	9	50	50	A
*T. serialis (N16623)	0	0	18	10	
*T. serpens (NF7343)	65	25	45	37	
*T. subrosea (B833)	2	4	27	20	
*T. variiformis (NF8046)	0	0	34	25	
*T. variiformis (N16573)	5	0	40	40	A
*T. variiformis (N16608)	33	5	18	15	A
*T. variiformis (B835)	26	14	30	25	A
Ungulina betulina (L333)	7	7	55	55	A

Several types of inhibition were observed. In one type the bacterial streak ended in a sharp distinct line with no evidence of growth between this

line and the edge of the fungus colony (fig. 1A, 1B). In a second, isolated colonies of bacteria appeared between the break in the streak and the fungus colony. These colonies were presumably resistant to the antibacterial material produced by the fungus (fig. 1D, 1E). In a third, the growth of the bacteria gradually tapered out toward the fungus colony (fig. 1C). A fourth type was observed in which the growth of the bacteria was uniformly faint for some distance between the fungus colony and the region of heavy growth of the bacteria. Variations of these main types appeared. For example, there occurred a combination of tapering growth with resistant colonies. Most of these types of inhibition were reported previously by Robbins et al. (7).

In table 1 the maximum zones of inhibition are recorded regardless of the character of the inhibition zone. The fungi which produced inhibition zones characterized by the presence of scattered individual resistant colonies were *Drosophila caudata* (L278), *D. exalbicans* (L450), *D. fibrillosa* (CBS23, L15.2), *D. semivestita* (L281), *D. trepida* (L247), *D. vernalis* (L367), *Fomes spadiceus* (N8102), *Peniophora incarnata* (NF8090), *Pleurotus griseus* (NF2948), and *Polyporus fragilis* (N10227). Fungi which were found to produce inhibition zones of the third type were *Alnicola escharoides* (L205), *A. melinoides* (L206), *Collybia dryophila* (L109), *Crepidotus mollis* (L73), *Fomes applanatus* (N9295), *F. fraxinophilus* (NF1921), *Panaeolus campanulatus* (L371), *Schizophyllum commune* (N16654), and *Sparassis crispa* (N9244). Inhibition zones of the fourth type were found with *Clitocybe ditopa* (L357), *Coprinus picaceus* (L14), *C. plicatilis* (L461), *Deconica inquilina* (L54b), *Irpex cinnamomeus* (NF2169), *Peniophora pubera* (NF3456), *Polyporus abietinus* (N16634), *P. giganteus* (NF3769), *P. zonalis* (NF2049), *Poria albobrunnea* (N16574), *P. weirii* (N16606), and *Trametes lilacino-gilva* (NF7322).

The antibacterial activity of most of the fungi investigated increased with the age of the fungus colony. However, the activity of some decreased with age. For example, the colony of *Polyporus osseus* (N11701) inhibited *Staph. aureus* for a distance of 25 mm. when tested after 12 days; a 19-day-old culture inhibited *Staph. aureus* for 3 mm.

As was observed earlier (7) there seemed to be for the fungi included in this investigation a negative correlation between rapidity of growth and antibacterial activity. Most of the fungi which grew rapidly showed no inhibitory effects and most of those which were most active were more slowly

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Explanation of figure 1

A few types of inhibition obtained by the streak method. The plates were streaked with *Staph. aureus* (left) and *E. coli* (right). The colonies in A and B were grown on corn-steep agar, those in C, D, and E on thiamine peptone agar. A. *Drosophila subatrata* (L284.6), B. *Fomes annosus* (N8273), C. *Coprinus picaceus* (L14), D. *Drosophila fibrillosa* (L15), E. *Drosophila exalbicans* (L450). At the time of streaking the colony in A was eight days old. The other colonies were two weeks old.
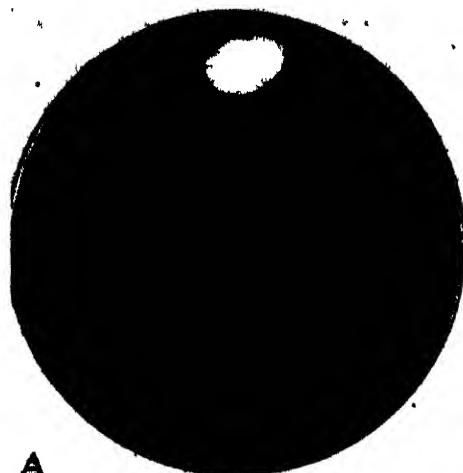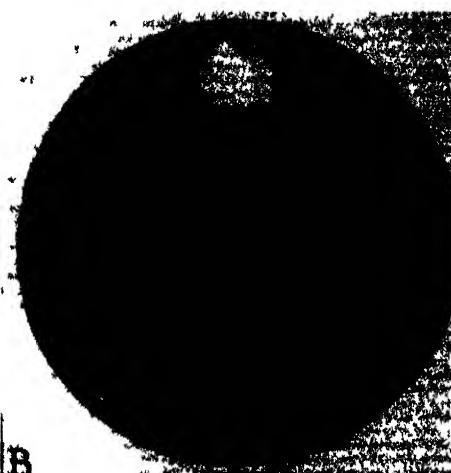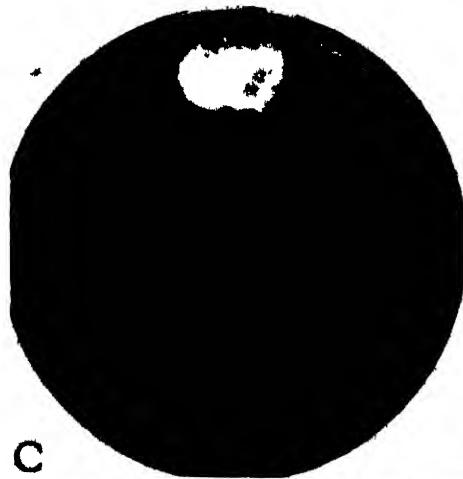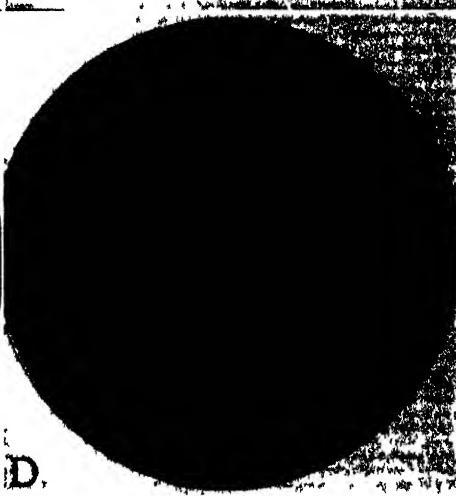
A B C D
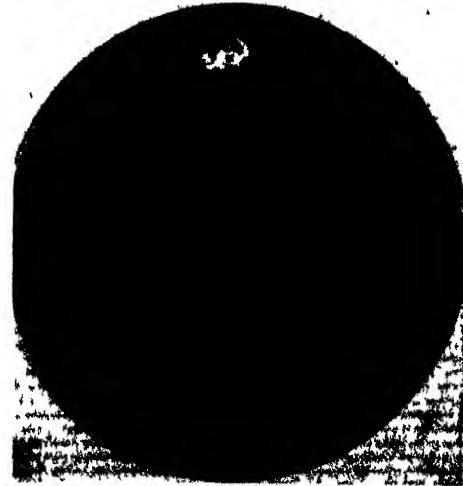
growing organisms. Furthermore, as discussed later, observations were made which suggested that a particular organism might produce more antibacterial material in media on which its growth was less rapid.

None of the fungi tested was active against *E. coli* alone. The antibacterial activity of 55 fungi was confined to *Staph. aureus* with none observed on *E. coli*. When a fungus was effective against both bacteria there was greater activity against *Staph. aureus* than against *E. coli*. In a few exceptions, when the inhibition zone was larger with *E. coli* than with *Staph. aureus*, the difference was slight or the inhibition zone for *E. coli* was partial. It would require further investigation to determine whether these exceptions were valid or the result of individual variation.

The medium upon which a fungus grew influenced the antibacterial activity to some extent. Six of the isolations tested evidenced antibacterial activity when grown on the corn-steep agar but none on the thiamine peptone agar. Thirty-five fungi displayed antibacterial activity when they were grown on the thiamine peptone medium but they were not effective when cultivated on the corn-steep agar.

Different isolations of the same species of some of the fungi were found to vary markedly in their antibacterial activity. For example, a culture of *Trametes serpens* (NF7343) displayed good activity against both indicator bacteria by the streak method. Another isolation of the same species (B) showed no activity. Two isolations of *Schizophyllum commune* (L445 and N11795) gave negative results with both bacteria. A third isolation of the same species (N16654) inhibited both *Staph. aureus* and *E. coli* on the thiamine peptone agar. A culture of *Poria albobrunnea* (B738) showed no antibacterial activity. Another isolation of the same species (N16574) inhibited both bacteria on both the corn-steep and thiamine peptone media.

Strain differences were also demonstrated for *Agrocybe semiorbicularis* (L92, L92.1, L92.6), *Deconica inquilina* (L54a, L54b, L54.27), *Drosophila appendiculata* (L20, L25, L25.8, L84, L108), *D. exalbicans* (L127, L127.4, L450), *D. gracilis* (L171.4, L460), *Fomes nigrolimitatus* (B708, N10085A, N16651, N16652), *Lentinus tigrinus* (L38.1, L38.2, L38.3, L38.11), *Poria weirii* (N9422, N16606, N16607), *Poria xantha* (N9315, N16570, N16571, N16629, N16630, N16645, N16647), *P. xantha crassa* (B805, B806, B807, B808), *P. xantha f. crassa* (B809, B810, B811), *Stereum abietinum* (N11792, N16603, N16604), and *S. sulcatum* (N8116, N16563, N16619).

Agar Disc Test. One hundred and eighty-three of the most active fungi listed in table 1 were selected for further study with the agar disc test. Each of these is marked by an asterisk in table 1. In addition, 23 of the fungi which showed little or no activity by the streak method were tested by the disc method. Each of these is marked by an asterisk on pages 479–485. The pro-

cedure used for the agar disc test was the same reported previously (7) except that each plate of yeast peptone agar[7] was seeded with 0.04 ml. of a 6–8 hour AC broth culture of the indicator bacterium (fig. 2). *Staph. aureus* was used as the indicator organism for all the fungi tested by the disc method; disc tests were run with *E. coli* also for those fungi which had showed marked inhibition of this bacterium by the streak test.

Results with the Agar Disc Test. The results for the fungi tested with *E. coli* as the indicator organism were almost entirely negative. Zones of inhibition were produced by the discs from only three species: *Agrocybe dura* (L386.10, L386.14), *Drosophila semivestita* (N281), and *D. subatrata*
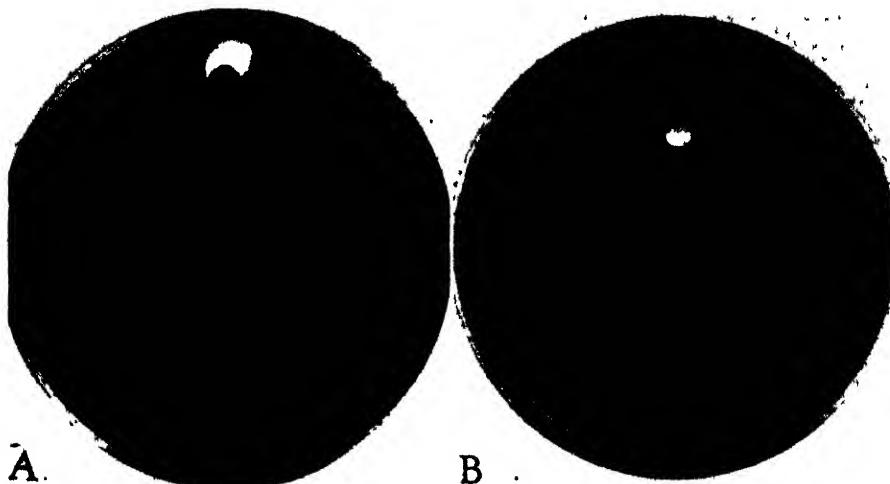
FIG. 2. The agar disc test with *Drosophila subatrata* (L284.6) grown on corn-steep agar for eight days. The discs cut from the culture in A were transferred to a yeast peptone medium seeded with *Staph. aureus*. The largest zone of inhibition in B was from the disc taken from nearest the center of the colony.

(L43.1, L284, L284.3, L284.6). The maximum inhibition zone was 9 mm. with *A. dura* and 15 mm. with *D. subatrata*. These zones showed partial inhibition, that is, contained numerous small colonies of bacteria.

Ninety fungi which had evidenced good activity by the streak test against *Staph. aureus* showed no activity against this bacterium by the agar disc test. Forty-eight isolations had some activity, producing zones with a maximum diameter of 10 mm.

The discs from 45 Basidiomycetes produced inhibition zones more than 10 mm. in diameter. The age and the medium on which the maximum zones of inhibition were produced by each of these fungi are recorded in table 2.

[7] The medium contained per liter 5 g. neopeptone, 3 g. yeast extract, 1 g. dextrose, and 15 g. Difco agar. The pH was 6.5 after autoclaving.

TABLE 2. *Antibacterial activity of fungi tested by the agar disc method.* The maximum inhibition zones in mm. against *Staph. aureus* are given. The age in days and medium on which the fungi were grown at the time of the test are indicated. C = corn-steep agar; P = thiamine peptone agar.

Organism	Medium	Age	Zone
Agrocybe dura (L386.10)	C	17	13
A. dura (L386.14)	P	23	11
Clitocybe obsoleta (L358)	P	13	22
C. robusta (NF2932)	C	16	11
Coprinus picaceus (L14)	P	12	20
Daelalea unicolor (N10202)	P	6	18
Drosophila caudata (L278)	C	12	13
D. exalbicans (L450)	C	23	15
D. fibrillosa (CBS23)	C	16	15
D. fibrillosa (L15)	P	17	17
D. fibrillosa (L15.2)	C	19	11
D. gracilis (L171.4)	C	28	14
D. semivestita (L281)	P	8	27
D. subatrata (CBS25)	O	16	28
D. subatrata (L43.1)	C	12	25
D. subatrata (L122)	C	13	25
D. subatrata (L284)	P	8	29
D. subatrata (L284.3)	C	13	27
D. subatrata (L284.6)	C	8	25
D. subatrata (L449)	P	10	28
D. vernalis (L367)	P	28	24
Fomes annosus (N8273)	C	20	16
Irpex mollis (NF3047)	C	16	13
Panaeolus papilionaceus (L387.3)	C	15	18
P. papilionaccus (L387.9)	C	13	15
Peniophora incarnata (NF8090)	C	17	19
Pleurotus griseus (NF2948)	C	16	15
Polyporus amorphus (N16597)	C	38	13
P. dichrous (N11609)	C	8	17
P. montanus (N8449)	C	20	14
P. obtusus (NF6798)	C	20	23
P. radiatus (N10229)	C	20	11
P. tephroleucus (?) (N10902)	C	20	14
Poria hypolateritia (N9249)	C	12	13
P. sequoiae (N8755)	C	17	12
P. sitchensis (B788)	P	9	12
P. subacida (N9762)	P	6	15
P. subacida (N16636)	C	10	20
P. versipora (B800)	C	9	15
Rhodopaxillus nudus (L352)	C	13	20
Stereum frustulosum (NF2055)	P	6	12
S. sulcatum (N8116)	C	17	12
Trametes americana (N9414)	C	10	15
T. heteromorpha (NF7343)	C	17	17
T. heteromorpha (N16558)	C	13	15

Various types of inhibition were observed (fig. 3). Some of these were described in the earlier report (7). Burkholder (3) has reported on some of these growth patterns of bacteria obtained from testing various antibiotic substances, vitamins, and amino acids in glass cylinders placed on plates seeded with bacteria.

The degree and character of antibacterial activity varied with the

fungus, the medium, the age of the cultures, and the position of the disc in relation to the fungus colony.

The zones of inhibition listed in table 2 were partial for *Clitocybe obsoleta* (L358), *Coprinus picaceus* (L14), *Drosophila gracilis* (L171.4), *Polyporus amorphus* (N16597), *Poria subacida* (N9762, N16636), *P. versipora* (B800), and *Stereum sulcatum* (N8116). The fungi producing inhibition zones containing individual resistant bacterial colonies were *Drosophila exalbicans* (L450), *D. fibrillosa* (CBS23, L15), *D. vernalis* (L367), *Fomes annosus*
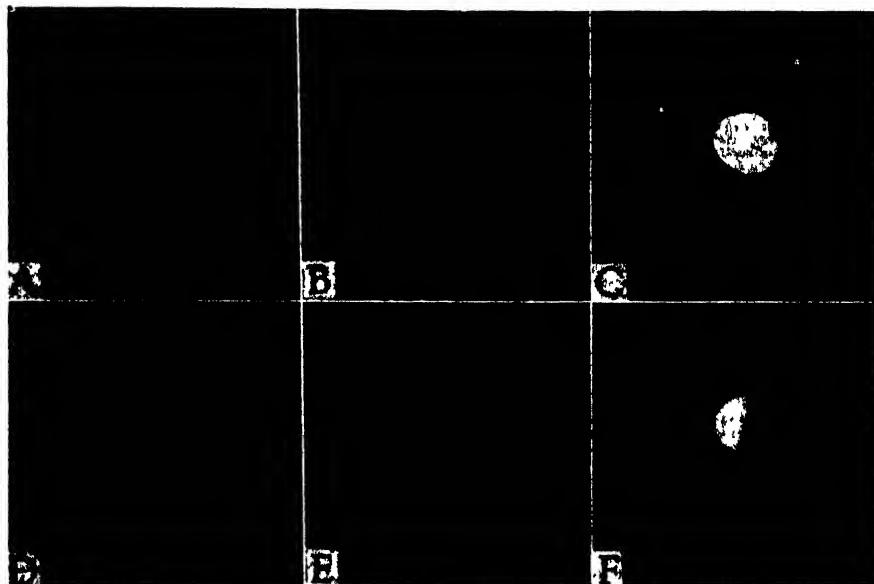
FIG. 3. Types of inhibition zones obtained by the agar disc method. The fungi were grown on corn-steep agar with the exception of *Drosophila subatrata* (L284.6) which was cultivated on the thiamine peptone agar. The disc in C was taken from the fungus colony; the discs in A, B, D and E were cut from outside the limits of the colony; half of the disc in F is from the colony. A. *Drosophila subatrata* (L284.6), B. *Fomes annosus* (N8273), C. *Drosophila fibrillosa* (L15), D. *Drosophila subatrata* (L449), E. and F. *Poria subacida* (N9762). The zone in A shows complete inhibition of *Staph. aureus;* B and C show types of inhibition zones characterized by scattered individual resistant colonies and partial inhibition; in D a zone of stimulation surrounds the inhibition zone; E shows alternating zones of more or less marked inhibition; in F a zone of partial inhibition surrounds the zone of marked inhibition; the inhibition zone in F is asymmetrical with greater inhibition on the side of the disc containing mycelium.

(N8273), *Panaeolus papilionaceus* (L387.3, L387.9), *Polyporus tephroleucus* (?) (N10902), *Rhodopaxillus nudus* (L352), and *Trametes americana* (N9414). The zones produced by discs from the other fungi tested were clear.

With most of the fungi tested the greatest activity was produced by the disc taken from nearest the center of the fungus colony. With a few of the fungi tested, for example, *Poria versipora* (B800) and *P. subacida* (N16636),

the disc with greatest activity was the one with the youngest mycelium, that taken from the periphery of the colony. The discs from just outside the limits of the colony of *Agrocybe dura* (L386.14) grown on the thiamine peptone agar for 23 days produced larger inhibition zones than those from within the mycelial colony. The antibacterial activity of *Clitocybe robusta* (NF2932) and *Pholiota lucifera* (NF673) was confined to the discs taken from the colonies. The differences in the activity of discs cut from various parts of the fungus colony or outside of it are doubtless correlated with the stability of the antibacterial substances, the readiness with which they are excreted by the fungus, the rate at which they diffuse in the agar, and other factors which were not analyzed in this study.

Correlation between the Streak and Disc Tests. As noted in the previous report (7) the correlation between the results obtained from the streak method and the disc method was not always satisfactory. A fungus which displayed good antibacterial activity by the streak test might evidence little or no activity by the disc test. Various explanations for this discrepancy were considered and some of them were tested experimentally. In the streak test we are dealing with materials produced by an entire fungus colony which has been growing for several days or even weeks. Whereas in the disc test the antibacterial material must be sufficiently concentrated in and diffusible from an agar disc 5.5 mm. in diameter to produce its effect within a few hours.

Trametes serpens (NF7343) evidenced marked activity by the streak method when grown on the corn steep medium. Discs of various sizes cut from the fungus colony were tested against *Staph. aureus*. Practically no inhibition was observed for discs 5.5 mm. in diameter, but discs 15 mm. in diameter showed distinct inhibition of the bacteria, and the size of the inhibition zones was directly correlated with the size of the discs.

Another factor considered was the slowness of diffusion of the antibacterial substances. The activity of discs placed on a yeast peptone agar seeded with *Staph. aureus* and kept at 15° C for 4.5 hours before incubation at 37° C was compared with that of similar discs incubated immediately at 37° C.

For six fungi tested in this way there was no difference in the activity of the discs incubated immediately and those from which the material was permitted to diffuse before incubation. For others the inhibition shown was greater for the discs first kept at 15° for 4.5 hours. For example, an agar disc from *Merulius lachrymans* kept at 15° C for 4.5 hours before incubation at 37° produced an inhibition zone measuring 19 mm., as compared with a zone of 11 mm. produced by a similar disc on a plate incubated immediately at 37° C.

Another factor involved was the test medium. In the streak test the indicator organisms were grown on the thiamine peptone or corn-steep medium on which the fungi were cultivated. The disc tests were carried out on a yeast peptone medium seeded with the indicator bacteria.

The effect of the test medium was determined for twenty fungi which gave inhibition zones of at least 10 mm. when streaked with *Staph. aureus.* Agar discs from 23-day-old cultures grown on the corn-steep and thiamine peptone media were placed on three media equally seeded with *Staph. aureus.*

TABLE 3. *Effect of the test medium on antibacterial activity by the agar disc method.* The organisms were grown for 23 days on the corn steep and thiamine peptone agar. Similar discs were tested on three media equally seeded with *Staph. aureus.* C = corn-steep agar; P = thiamine peptone agar; Y = yeast peptone agar. Maximum inhibition zones in mm.

Organism	Grown on C			Grown on P		
	Discs tested on					
	C	P	Y	C	P	Y
Clitocybe robusta (NF2932)	30	25	15	22	18	9
Coniophora puteana (N9779)	11	15	0	0	7	0
Coprinus comatus (NF2935)	9	8	0	15	12	0
Irpex mollis (NF3047)	12	11	13	16	14	11
Lenzites bicolor (NF7326)	0	0	7	0	0	0
Merulius lachrymans (NF3526)	10	7	0	24	22	11
Paxillus panuoides (NF3007)	8	11	0	10	11	0
Pholiota lucifera (NF673)	18	18	11	15	19	8
Pleurotus griseus (NF2948)	16	13	12	11	8	7
Polyporus amorphus (N8552)	16	15	0	9	10	0
P. caesius (NF6891)	9	0	0	0	0	0
P. semipileatus (NF3499)	20	10	8	15	14	8
Trametes carbonaria (N8432)	12	9	0	8	8	0
T. serpens (NF7343)	15	8	6	0	0	0
T. variiformis (NF8046)	7	0	0	0	0	0

The three media included the corn-steep, thiamine peptone, and yeast peptone agars. The maximum inhibition zones in millimeters obtained from a series of three discs cut on a radius starting at the center of the colony are recorded in table 3.

The activity of the discs was greatest for most of the fungi tested on the corn-steep test medium and least on the yeast peptone medium. This may be associated with the higher pH of the yeast peptone medium[8] or the fact that it is better for the growth of *Staph. aureus* than the corn-steep medium. However, this was not true with all the fungi. A disc from a culture of *Lenzites bicolor* (NF7326) which gave no activity on the corn-steep or thiamine peptone media showed slight activity on the yeast peptone agar. The activity of the discs from cultures of *Coniophora puteana* (N9779) and

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[8] The pH of the yeast peptone agar was 6.5 as compared with a pH of 5.7 for the corn-steep agar and a pH of 5.5 for the thiamine peptone agar.

Pholiota lucifera (NF673) was best on the thiamine peptone test medium. The discs from cultures of *Corticium portentosum* (N9283), *Omphalia flavida* (NF5711), *Polyporus mollis* (NF6827), *P. nidulans* (N9312), and *Poria candidissima* (N11613) were inactive on the three test media.

It was observed that the test medium sometimes affected the character of the inhibition zone. For example, discs from a culture of *Merulius lachrymans* (NF3526) grown on the thiamine peptone agar produced clear zones on the yeast peptone agar, but produced larger zones of partial inhibition on both the corn-steep and thiamine peptone media.

The three test media used above varied in their sugar content. The corn-steep agar contained 40 g. of dextrose per liter; the thiamine peptone agar 10 g. per liter; the yeast peptone one gram per liter. By increasing the dextrose in this medium to 40 g. per liter the activity of the discs of *Clitocybe robusta* (NF2932) and *Polyporus semipileatus* (NF3499) on the yeast peptone agar was increased. The inhibition zones on the yeast peptone agar plus added dextrose, however, were not so large as the zones produced by comparable discs on the corn-steep test medium. Substances other than sugar alone are evidently concerned with the differences obtained with the various test media.

It seems probable that no single explanation can account for all those instances in which marked activity was observed by the streak test and little or none by the disc test and the relative importance of the different factors probably varies with the fungus. In general the disc test on the yeast peptone medium puts the antibacterial substances to a more severe test.

A few fungi which displayed negative or almost negative antibacterial activity by the streak test gave some activity with the disc test. For example, discs cut from the colony of *Lenzites saepiaria* (N9528) grown on the corn-steep agar for 20 days produced inhibition zones as large as 13 mm. in diameter with *Staph. aureus*. Similar observations were made with *Polyporus tulipiferus* (NF8049). It appears that the active material was largely retained by the mycelium and released upon the cutting of the discs.

Liquid Cultures and the Serial Dilution Method. Fifty-three of the fungi found to have the most antibacterial activity by the streak and disc tests were grown in liquid culture. Each fungus was usually grown at 25° C in Fernbach flasks, each containing one liter of medium and 4–6 coils of beech shavings. The corn-steep and thiamine peptone media used for the streak and disc tests were prepared without agar. When the mycelial mat had grown on the surface of the liquid a sample of the culture fluid was assayed against *Staph. aureus* by the serial dilution method used in this laboratory (5).[9]

Samples of the culture fluids were taken at intervals depending upon the rapidity of growth of the fungus. This was usually after a 1–4-week period. If the fluid was active, another sample was usually taken in a few days or a week later. If it was apparent that there was no further increase in titer with age, the culture fluid was poured out of the flask and a liter of fresh sterile medium was added so that the mycelial mat could come in contact with it. For some of the most active fungi only a few days contact was necessary in order to obtain a culture fluid with suitable antibacterial activity. A mat could be reflooded several times by this method.

The following fungi grown on the corn-steep or thiamine peptone media produced fluids with a maximum activity of 32 dilution units or less against *Staph. aureus:*

Agrocybe dura (L386.14)	*P. dichrous* (N11609)
Clitocybe robusta (NF2932)	*P. mollis* (NF6827)
Coniophora puteana (N9779)	*P. montanus* (N8449)
Coprinus comatus (NF2935)	*P. nidulans* (N9312)
C. picaceus (L14)	*P. obtusus* (NF6798)
Corticium portentosum (N9283)	*P. radiatus* (N10229)
Drosophila fibrillosa (L15, L15.2)	*P. semipileatus* (NF3499)
D. vernalis (L367)	*Poria candidissima* (N11613)
Irpex mollis (NF3047)	*P. sequoiae* (N8755)
Lenzites bicolor (N7326)	*P. subacida* (N9762)
Merulius lachrymans (NF3526)	*Stereum frustulosum* (NF2055)
Omphalia flavida (NF5711)	*S. sulcatum* (N8166)
Panaeolus papilionaceus (L387.3, L387.9)	*Trametes americana* (N9414)
Paxillus panuoides (NF3007)	*T. carbonaria* (N8432)
Peniophora incarnata (NF8090)	*T. heteromorpha* (N16558)
Pholiota lucifera (NF673)	*T. serpens* (NF7343)
Polyporus amorphus (N8552)	*T. variiformis* (NF8046)
P. caesius (NF6891)	

Culture fluids with activity of 64 dilution units or more were obtained from sixteen isolations as recorded in table 4. The second column gives the maximum activity in dilution units against *Staph. aureus*. The third column shows whether the greatest activity was obtained with the corn-steep (C) or thiamine peptone (P) medium. The fourth column records the type of container in which the cultures were growing at the time of the maximum activity of the culture fluid. These were either Fernbach flasks (F) containing one liter of the solution and beech shavings or 250-ml. wide-mouthed Erlenmeyer flasks (250) each with 100 ml. of the medium and beech shavings. The last column gives the age of the culture fluid at the time its greatest activity was observed. The most active culture fluids obtained with three of the fungi were the result of reflooding the mycelial mats. The culture of *Trametes heteromorpha* (N8440) was reflooded with fresh sterile solution after one month's growth. The mats of *Clitocybe obsoleta* (L358) and *Rhodopaxillus nudus* (L352) had grown a little more than three months before reflooding.

TABLE 4. *Antibacterial activity of fungi grown in liquid cultures.* Maximum activity in dilution units. C = corn-steep liquid; P = thiamine peptone liquid; F = Fernbach flask; 250 = 250 ml. wide-mouthed Erlenmeyer flask. See text for details.

Organism	Maximum activity dilution units	Medium	Container	Age
Agrocybe dura (L386.10)	512	C	F	6 weeks
Clitocybe obsoleta (L358)	128	C	250	6 week reflood
Daedalea unicolor (N10202)	256	P	250	4 weeks
Drosophila semivestita (L281)	64	C	F	4 weeks
D. subatrata (CBS25)	2048	C	F	2 months
D. subatrata (L43.1)	1024	C	F	4 weeks
D. subatrata (L122)	256	C	F	7 weeks
D. subatrata (L284)	64	C	F	5 weeks
D. subatrata (L284.3)	1024	C	F	4 weeks
D. subatrata (L284.6)	2048	C	F	5 weeks
D. subatrata (L449)	64	C	F	2 months
Fomes annosus (N8273)	128	C	250	12 days
Poria hypolateritia (N9249)	128	C	250	16 days
P. sitchensis (B788)	64	C	250	16 days
Rhodopaxillus nudus (L352)	1024	C	F	16 day reflood
Trametes heteromorpha (N8440)	64	C	F	17 day reflood

Very active culture fluids were obtained from several isolations of *Drosophila subatrata.* Culture fluids active at a dilution of 1 to 2048 were obtained with CBS25 and L284.6. A 20-day-old mycelial mat (L284.6) reflooded with the corn-steep medium produced a liquid active at a dilution of 1 to 1024 after six days.

A 48-day-old mycelial mat of *Agrocybe dura* (L386.10) produced a culture liquid active at a dilution of 1 to 256 ten days after reflooding with the corn-steep liquid. Reflooding a mycelial mat of *Rhodopaxillus nudus* (L352) which had previously grown for three months in a 250-ml. flask with the corn-steep liquid produced a fluid active at a dilution of 1 to 512 after five days.

Trametes serpens (NF7343). In the course of this study it was noted that several fungi showing excellent antibacterial activity by the streak test produced disappointing results by the disc test. One of the fungi which appeared to be particularly worthy of further investigation was *Trametes serpens* (NF7343). When this organism was grown on the corn-steep agar, inhibition zones by the streak test were observed measuring 65 mm. on *Staph. aureus,* 25 mm. on *E. coli,* and 20 mm. on *Pseudomonas aeruginosa.* On the thiamine peptone agar inhibition zones of 45 mm. on *Staph. aureus,* 37 mm. on *E. coli,* and 20 mm. on *P. aeruginosa* were obtained. The growth on the corn-steep medium was very slow, the radius of the colony measuring about 3 mm. after two weeks. Growth on the thiamine peptone agar was more rapid, the radius measuring 22 mm. after two weeks. A very slight amount of

acid was produced by the fungus. On a tryptose[10] agar the fungus inhibited *Mycobacterium phlei* for 25–28 mm. However, colonies showing good inhibition by the streak test gave negative results with the disc test.

How far the composition of the medium on which the fungus was grown affected the antibacterial activity against *Staph. aureus* was determined by the agar disc test. A variety of natural media and synthetic media supplemented in different ways was used. The pH of some of the media was adjusted to determine the effect of hydrogen ion concentration. A preliminary experiment indicated that the organism grew best at 25° C and this temperature was used throughout the experiments described below.

The corn-steep agar was varied by substituting K_2HPO_4 for the KH_2PO_4 in order to make the medium more alkaline. This medium was also used with the addition of 2 ml. potato extract[11] per l. Agar discs were cut from colonies 5 weeks old. The potato extract reduced the growth of the organism on the alkaline corn-steep agar; the colony was compact, brownish, with a radius of 7 mm. The maximum inhibition zone from discs taken from this culture was 14 mm. in diameter and a disc cut more than 20 mm. from the edge of the colony produced an inhibition zone of 11 mm. On the alkaline corn-steep agar the mycelium was white and the colony radius was 25 mm. The maximum inhibition zone by the disc test was 10 mm. and the activity was confined to the discs with mycelium.

Trametes serpens was grown on various media in Petri dishes to determine the effect of medium on growth and antibacterial activity. Supplementary observations were made on the growth of the fungus on the various media in test tubes. All media were prepared with 15 g. Difco agar per l. These included (a) 2 per cent malt extract, (b) 2 per cent malt extract treated with Norit A, (c) 5 per cent malt extract, (d) 2 per cent malt extract to which was added malt eluate[12] equivalent to 3 per cent malt extract, (e) a thiamine peptone stock culture medium containing per liter 1.5 g. KH_2PO_4, 0.5 g. $MgSO_4$ $7H_2O$, 50 g. dextrose, 1 g. neopeptone, 2 g. asparagine, 0.5 ml. mineral supplements, and 500 mμmoles thiamine, (f) a tryptose medium containing per liter 10 g. Bacto tryptose, 10 g. dextrose, 2.5 ml. of a vitamin mixture,[13] and 1.25 γ biotin, (g) the same with malt eluate, (h) the AC medium (see 5), (1) the same plus malt eluate equivalent to 5 per cent malt extract, (j) a basal medium containing per liter 1.5 g. KH_2PO_4, 0.5 g. $MgSO_4$ $7H_2O$, 50 g. dextrose, 2 g. asparagine, 0.5 ml. mineral supplements,

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[10] The medium contained per liter 20 g. Bacto tryptose, 10 g. dextrose, 15 g. Difco agar, 2.5 ml. of a vitamin mixture (see footnote 13), and 1.25 γ biotin.

[11] The dry weight of the potato extract was 594.8 mg./ml.

[12] The malt eluate was prepared by extracting the Norit A with ammoniacal acetone.

[13] The mixture contained per ml. 100 mμmoles each of p aminobenzoic acid, calcium pantothenate, guanine, hypoxanthine, 2 methyl-1,4 naphthohydroquinone diacetate, pimelic acid, nicotinamide, pyridoxine, riboflavin, thiamine, and 100,000 mμmoles i-inositol.

12 ml. of a vitamin mixture,[13] 6 γ biotin, and 625 mg. casein hydrolysate (the pH was raised to 5.8 with NaOH), (k) the same with added Hutchings synthetic casei factor (Lederle) 120 γ per liter, (l) k with 1200 γ folic acid (R. J. Williams π 4000) per liter.

The growth of the fungus was best on medium (a) and least on (e), (h), (i), (j), (k), and (l). Colonies grown for 16 days in Petri dishes on these different media were tested against *Staph. aureus* using the agar disc method. Negative results were obtained from colonies grown on (c), (h), and (i). The largest and clearest zones of inhibition, measuring 19 mm. in diameter as a maximum, were obtained from colonies grown on (j), (k), and (l). The zones from (b) were as large but the inhibition was partial.

Since medium (j) appeared to be the most satisfactory for the production of antibacterial activity, further experiments were carried on with this medium in which the sources of nitrogen and amounts of dextrose were varied. The nitrogen sources used at 1, 2, and 4 g. per liter included casein hydrolysate, asparagine, neopeptone, synthetic gelatine hydrolysate, a mixture of five amino acids,[14] and another mixture of six amino acids.[15] The amounts of dextrose used were 10, 20, and 50 g. per liter.

The growth of *Trametes serpens* was best with the various amounts of the synthetic gelatine hydrolysate and with 4 g. neopeptone per liter. After two weeks, agar discs from the colonies grown on the various media were tested against *Staph. aureus* by the agar disc test. Negative or nearly negative results were obtained from the colonies grown on both mixtures of amino acids and the synthetic gelatine hydrolysate. Zones with partial inhibition were observed for the discs from the colonies grown on the casein hydrolysate and neopeptone. The largest and clearest inhibition zones were obtained from the cultures grown on the asparagine media. The best medium for antibacterial activity appeared to be the basal medium prepared with 2 g. asparagine and 50 g. dextrose per liter. When this medium was used, an inhibition zone 21 mm. in diameter was produced. The antibacterial material diffused readily in sufficient concentration so that a disc cut more than 20 mm. from the edge of the fungus colony produced an inhibition zone 10 mm. in diameter. The growth of the fungus on this medium was slow, the colony radius measuring 3 mm. after two weeks.

These experiments on the effect of the culture medium showed that, in general, the greatest antibacterial activity was obtained on those media on which the fungus grew slowly. The asparagine medium appeared to be the most satisfactory. A culture fluid with an activity of 64–128 dilution units against *Staph. aureus* was obtained after one month's growth of the fungus

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[14] These included d-arginine, dl-alanine, dl-aspartic acid, glycine, and d glutamic acid.
[15] These included l-leucine, d-isoleucine, dl-phenylalanine, d-glutamic acid, l-tyrosine, d-arginine, and were prepared so that they were in the same proportions as in casein.

in the liquid asparagine medium in a 500-ml. Erlenmeyer flask or in a 250-ml. wide-mouthed flask.

Since *Trametes serpens* grew best on a 2 per cent malt agar but showed its greatest antibacterial activity on the synthetic medium described above prepared with 2 g. asparagine and 50 g. dextrose per liter, it was hoped that an active culture fluid would be obtained by growing the fungus in a 2 per cent malt liquid until the formation of a thick mycelial mat and then replacing the malt liquid with the asparagine medium. The results were disappointing. The highest activity obtained by reflooding a mycelial mat grown in a Fernbach flask with the asparagine medium was 16 dilution units. Culture fluids with a maximum activity of 16 dilution units were also obtained by reflooding mats previously grown on 2 per cent malt with the corn-steep medium, a corn-steep medium prepared with K_2HPO_4, and the same with added potato extract (2 ml. per l.).

DISCUSSION

Antibacterial activity was found to be widespread in the group of Basidiomycetes included in this investigation. About 55 per cent of the 508 isolations investigated by the streak method showed antibacterial activity against *Staph. aureus* or against *Staph. aureus* and *E. coli*. This percentage of active isolations compares favorably with those of other investigations on the Basidiomycetes and other groups of fungi. Strict comparisons would not be valid, considering the difference in methods used by the various investigators.

Only 17 of the 64 genera included in this survey demonstrated no antibacterial activity by the streak method and 14 of these genera were represented by a single species. For 6 genera, each represented by 29 or more isolations, the number of isolations which were negative, moderately active or strongly positive is given in table 5, which includes in the last column the number observed to produce acid which might account for the antibacterial activity observed. Of the six genera, *Drosophila* and *Trametes* showed the largest

TABLE 5. *Distribution of antibacterial activity in 6 genera surveyed by the streak method.* A rating of strongly positive was given fungi giving inhibition zones of more than 10 mm. on *Staph. aureus.*

Genus	Total number of isolations	Negative	Moderately active	Strongly positive	Acid producers
Coprinus	38	31	2	5	0
Drosophila	48	16	9	23	0
Fomes	40	19	6	15	6
Polyporus	94	51	8	35	12
Poria	80	31	16	33	17
Trametes	29	5	5	19	10

proportion of active isolations. However, a considerable number in these genera were isolations of the same species.

It need not be pointed out that the results obtained on antibacterial activity depend on the methods of investigation as well as the fungi investigated. The streak test has definite limitations. It does not detect antibacterial substances produced and retained within the mycelium. The number of fungi among those investigated which produced intracellular antibacterial substances was not determined by the method of survey used. Observations on *Lenzites saepiaria* (N9528) and *Polyporus tulipiferus* (NF8049), which were negative by the streak test but positive by the disc test, in which the injury to the mycelium in cutting the discs might permit intracellular antibacterial substances to diffuse, emphasize the limitation of the streak method discussed here.

It is possible that antibacterial activity might have been found in a number of the fungi included in this investigation if extracts of the mycelium had been made and tested for activity against the indicator bacteria. However, from a practical standpoint, that is, the production of sufficient antibacterial material to permit concentration and possible isolation, the intracellular substances are of less importance than the extracellular. It would be more difficult to grow sufficient mycelium for extraction purposes than to deal with culture liquid.

The streak method is further complicated because of the effect of hydrogen ion concentration, resulting from organic acids produced by the metabolism of the fungus and by the possible exhaustion of nutrients essential for the growth of the bacteria in the vicinity of the fungus colony. The hydrogen ion concentration was taken into account by suitable tests at the time the plates were streaked. However, the possible importance of the exhaustion of essential nutrients in the vicinity of the colony could not be estimated in this investigation by the streak method. It should be pointed out, however, that neither of these factors is of importance when the disc test is used, because the yeast peptone agar upon which the discs were placed furnishes an adequate medium for the indicator bacteria and because its buffer action neutralizes the small amount of acid which might diffuse from the agar disc.

The medium on which the fungus is grown is another factor of importance in determining its antibacterial activity. This was illustrated by the studies on *Trametes serpens* and by the comparisons between corn-steep agar and thiamine peptone agar for a number of fungi investigated. It is possible that some of the Basidiomycetes included in this study would evidence activity if cultivated on some other medium. In this investigation the two media on which all organisms were tested were chosen because they permitted growth of the fungi and of the indicator bacteria, thus avoiding the

necessity of coating the agar on which the fungi had grown with a medium suitable for the growth of the indicator bacteria.

Evidence of antibacterial activity depends also upon the indicator organisms. *Staph. aureus* and *E. coli* represented the Gram-positive and Gram-negative bacteria as in the work of most other investigators. It is recognized, however, that because of the specificity in the action of antibacterial substances, the best indicator organism to employ is the one for which an antibiotic substance is desired. It would be desirable to re-survey the fungi included in this investigation using other indicator bacteria, especially those belonging to the acid-fast group.

The age of the fungus colony is also important in an investigation of antibacterial activity. Most of the organisms were tested at two ages. These results show that antibacterial activity may disappear with age, or it may increase with age, depending upon the fungus and the conditions under which it is grown. Here again, it is possible that more extensive search than time permitted might change some fungi from the negative to the positive class.

The results depend also on the strain of the fungus investigated. This has been demonstrated by others for various fungi including *Penicillium notatum* and other species found to produce antibiotic substances. Differences in the antibacterial activity among several isolations of the same species were also found in this survey. Certain isolations of *Trametes serpens, Schizophyllum commune,* and *Poria albobrunnea* were inactive. Other isolations of these same species evidenced antibacterial activity. Strain differences were also demonstrated for eleven other species included in this report.

When results of the present survey are compared with those of other investigators, differences are found. The present survey included 66 species reported on by Wilkins and Harris (12). Nineteen of these were negative according to both studies. Six species found active by Wilkins and Harris gave negative results in this survey. Twenty-nine reported by Wilkins and Harris to be negative displayed varying degrees of antibacterial activity in this survey. Sixteen of the species reported on by Mathieson (6) were included in this report. Five species which he found negative gave zones of inhibition of more than 10 mm. by the streak method. The results with the other 11 species were similar in both reports. Fifty-three species reported here were surveyed by Wilkins (9). Of 9 species with strongly positive results in his report, one was negative in this survey. Of 33 species found by Wilkins to be negative, 7 were found to be active. Of 76 species reported by Robbins and his associates (7) to give negative results, 13 were found to produce inhibition zones of more than 10 mm. in this investigation. Seven of the 58 species found by Robbins and associates to produce inhibition zones of more than 10 mm. gave negative results in this survey.

Differences in antibacterial activity found by various workers may be accounted for to some extent by differences in the fungi employed. There is no guarantee that the organisms studied under a particular species name were identical as to species or strain. In addition, the methods of investigation vary. For example, Wilkins and Harris and Mathieson used extracts of the sporophores for their investigations; Robbins and his colleagues used the streak method but the fungi were grown on the thiamine peptone medium and a 2 per cent malt agar. To evaluate the relative importance of fungi and methods in determining the differences in the results of various investigators it would be necessary to obtain subcultures of the particular isolations used in one laboratory and study them in another.

A number of the Basidiomycetes surveyed in this paper seem to be worthy of further investigation, in particular, *Drosophila subatrata*, *Rhodopaxillus nudus*, and *Agrocybe dura*. Whether or not any of them became therapeutically important can only be determined by further research.

SUMMARY

About 55 per cent of the 508 isolations of Basidiomycetes investigated by the streak method showed antibacterial activity against *Staph. aureus* or against *Staph. aureus* and *E. coli*. None was active against *E. coli* alone. Two hundred and three inhibited the growth of *Staph. aureus* for more than 10 mm. by this method. For about 25 per cent of these, the inhibition was probably caused by the hydrogen ion concentration. Forty-five isolations produced inhibition zones more than 10 mm. in diameter by the agar disc method. Culture fluids with activity of 64 dilution units or more were obtained from sixteen isolations. Culture fluids active at a dilution of 1024–2048 were obtained with several isolations of *Drosophila subatrata* and *Rhodopaxillus nudus*. With several fungi there appeared to be a negative correlation between growth and antibacterial activity.

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OBSERVATIONS ON STREPTOMYCES GRISEUS—I. CHEMICAL CHANGES OCCURRING DURING SUBMERGED STREPTOMYCIN FERMENTATIONS

EUGENE L. DULANEY AND D. PERLMAN

In order to control a fermentation it is helpful, if not necessary, to understand its mechanism; these studies of the various factors influencing streptomycin production have therefore included an investigation of the chemical changes occurring during the fermentation.

A review of the literature indicates that knowledge of the metabolic activities of the actinomycetes in surface or submerged culture is quite limited. Schatz and his co-workers (1944) and Waksman and others (1946) have described in some detail the changes occurring during the production of streptomycin in surface culture but did not investigate the submerged fermentation so extensively. Somewhat earlier Woodruff and Foster (1943) reported a few of the changes occurring during the production of streptothricin and also actinomycin. The following experiments were conducted preparatory to investigating other factors governing the course of the fermentation on natural and synthetic media.

METHODS .

Cultural: An isolate from a culture originally obtained from Dr. S. A. Waksman's laboratory was used in these studies. Stock cultures were maintained by mixing spores with sterile soil. Inoculum was developed by spreading spores from the soil tubes over a Blake bottle slant of yeast extract—glucose agar medium. These cultures were incubated at 28° C until sporulation had occurred; cultures not used immediately were refrigerated. Fifty ml. of sterile distilled water were added to each bottle and a spore suspension was prepared by scraping with a sterile wire needle. One ml. of this spore suspension served as inoculum for each fermentation flask.

Unless otherwise indicated the basal medium contained 10 g. glucose, 5 g. peptone, 5 g. sodium chloride, 5 g. meat extract, and sufficient distilled water to bring the volume to 1 l. This is the medium used by Waksman and his co-workers (1946). Forty ml. aliquots were distributed in 125 ml. Erlenmeyer flasks, the flasks were plugged with cotton, and sterilized at 121° C for 20 min. They were inoculated with the spore suspension when cool and then placed on a rotary shaker. The shaker imparted a circular motion to the flasks such that all points on the flask described a circle of 1.5 in. diameter; the speed was 220 rpm. This shaker was located in a

constant temperature room maintained at 28° C. At the end of each fermentation interval two or three replicate flasks were removed and the contents pooled for analysis.

Analytical: The mycelium was removed from an aliquot of the fermented medium and the cell-free solution was analyzed for the following substances: Soluble nitrogen by a modification of Johnson's method (1941); glucose (residual reducing substance) by the method of Shaffer and Somogyi (1933); inorganic phosphorus by a modification of the procedure of Fiske and SubbaRow (1925); soluble carbon by a micro adaptation of the method of Friedemann and Kendall (1929).

Aliquots of tne whole culture were analyzed for: ammonia nitrogen by the procedure of Gailey and others (1946); lactic acid by Barker and Summerson's method (1941); streptomycin by a modification of the method outlined by the Food and Drug Administration (1945) with pure streptomycin as a reference standard; oxygen demand (Q_{O_2} per ml. of medium) was determined as described by Koffler and his co-workers (1945). The pH was measured with a glass electrode. A ten ml. aliquot was placed in a tared test tube and centrifuged. The collected mycelium was resuspended in distilled water to remove any remaining media and centrifuged a second time. The collected mycelium was then dried to constant weight at 100° C (12–16 hrs.); replicate determination agreed within 3 per cent. The dried mycelium was analyzed for nitrogen by Johnson's micro-Kjeldahl method (1941).

EXPERIMENTS

The chemical changes occurring during the fermentation of the basal medium are presented in table 1. These analyses indicated that the medium

TABLE 1. *Changes Occurring During Fermentation of Glucose—Meat extract—Peptone Medium.*[a]

	Duration of fermentation (days)								
	0	1	2	3	4	5	6	7	8
pH	7.35	7.30	7.55	7.50	7.75	8.25	8.55	8.65	8.90
Mycelium mg./ml.		0.4	5.1	5.8	5.7	4.8	4.6	4.2	3.8
Streptomycin γ/ml.		0	37	194	198	231	270	186	267
Glucose mg./ml.	9.0	8.8	8.0	2.4	1.2	0.6			
Soluble C mg./ml.	10.2	8.6	7.0	5.1	5.0	4.4	4.6	4.5	4.6
Lactic acid γ/ml.	292	328	114	13	10	16	12	6	15
Oxygen (Q_{O_2}/ml.) demand		19	81	82	53	25	−5		
Soluble N mg./ml.	1.48	1.30	1.10	0.67	0.70	0.73	0.90	0.88	1.14
Mycelial N %		9.2	8.6	10.7	9.9	10.1	8.6	9.0	7.5
Inorganic P γ/ml.	118	108	34	1	5	2	19	24	34
NH₃-N γ/ml.	66	70	75	63	103	115	170	232	265

[a] Medium (per liter): 10 g. glucose; 5 g. meat extract; 5 g. peptone; 5 g. NaCl.

was very low in inorganic phosphate and that nearly all of the available phosphate was quickly assimilated by the organism. Accordingly a substantial quantity of inorganic phosphate was added to the medium to eliminate the possibility of this material acting as a limiting factor in the fermentation. Analyses of this supplemented medium are presented in table 2.

As the fermentable carbohydrate was very quickly utilized by the organism, it appeared possible that this might be a limiting factor in the fermentation. Various concentrations of glucose were added to the medium without marked effect on the production of streptomycin. It appeared that 1% glucose was nearly optimal under these fermentation conditions. The

TABLE 2. *Changes During Fermentation of High Phosphate Medium.*[a]

	Duration of fermentation (days)							
	0	1	2	3	4	5	6	7	8
pH	6.80	6.80	7.05	7.00	7.40	8.00	8.30	8.35	8.50
Mycelium mg./ml.		< 0.1	3.1	5.2	2.2	1.7	1.4	1.5	0.8
Streptomycin γ/ml.		0	0	25	18	79	71	55	51
Glucose mg./ml.	10.0	9.0	7.8	2.0	0.5	0.2	< 0.2		
Soluble C mg./ml.	11.4	10.8	9.4	6.9	4.8	5.1	5.0	4.9	5.2
Lactic acid γ/ml.	212	185	48	45	45	32	23	13	7
Oxygen (Q_{O_2}/ml.) demand		−10	+117	+104	+54	+30	+22	+20	+8
Soluble N mg./ml.	1.04	0.95	0.85	0.59	0.90	0.93	0.85	0.91	0.88
Mycelial N %			6.0	8.3	7.7	8.5	9.5	13.0	12.5
Inorganic P mg./ml.	1.35	1.31	1.18	1.08	1.21	1.21	1.20	1.30	1.38
NH_3-N γ/ml.	78	44	33	18	95	226	306	290	305

[a] Medium: 10 g. glucose; 5 g. meat extract; 5 g. NaCl; 5 g. peptone; 5 g. KH_2PO_4.

changes occurring during the fermentation of a high carbohydrate (3 per cent glucose) medium are presented in table 3.

DISCUSSION

The fermentation of this medium by *Streptomyces griseus* apparently falls into two phases as contrasted to the three phases during the *Penicillium chrysogenum* fermentation (Koffler *et al.* 1945). These general changes are summarized in table 4.

During the first or growth phase the mycelium is formed. This is accompanied by a marked reduction in the soluble nitrogen, the available inorganic phosphate, ammonia nitrogen, and fermentation of the available carbohydrate. During this phase the quantity of lactic acid present is first increased and then utilized to some extent. The oxygen demand is quite high during this first phase and Q_{O_2} values of 150 have been observed. It is interesting to note that during the period of germination of the spores a gas is evolved, thus giving a negative oxygen demand. This has been repeatedly

TABLE 3. Changes during Fermentation of High-Carbohydrate Medium.[a]

	Duration of fermentation (days)											
	0	1	2	3	4	5	6	7	8	10	11	12
pH	6.55	6.55	7.40	7.10	6.65	6.90	6.90	6.75	7.00	6.70	7.65	7.85
Mycelium mg./ml.		<0.2	3.3	7.1	5.5	4.9	4.5	4.8	3.8	3.6	3.6	3.1
Streptomycin γ/ml.		0	0	88	99	164	137	183	142	183	188	274
Glucose mg./ml.	30.0	29.0	27.0	23.0	19.0	16.0	10.6	8.5	4.5	2.5	2.0	1.5
Soluble C mg./ml.	19.8	19.6	17.0	14.3	14.0	12.2	7.5	6.7	6.3	6.5	5.7	5.9
Lactic acid γ/ml.	185	185	70	70	78	86	94	110	86	50	43	25
Oxygen (Q$_{O_2}$/ml.) demand	1.13	−12	+138	+112	+116	+70	+68	+46	+53	+39	+35	+26
Soluble N mg./ml.	1.13	1.07	0.75	0.53	0.64	0.58	0.61	0.64	0.58	0.78	0.73	0.80
Mycelial N %			8.1	5.5	6.3	7.8	9.5	9.0	10.0	10.2	10.5	9.8
Inorganic P γ/ml.	153	122	60	27	18	28	30	28	27	4	6	3
NH$_3$-N γ/ml.	37	16	55	13	16	<5	16	<5	16	19	101	120

[a] Medium (per liter): 30 g. glucose; 5 g. meat extract; 5 g. NaCl; 5 g. peptone.

observed and the period extends until most of the spores have germinated. Although the gas has not been positively identified it is not presumed to be carbon dioxide as the Warburg flasks contained potassium hydroxide in the center well. The soluble carbon content of the medium during the growth phase rapidly falls as the glucose is utilized. Approximately 50 per cent of the soluble carbon of this basal medium was apparently not available to the organism during the fermentation. It appears that the nitrogen content of the mycelium varies with the age.

During the second or autolytic phase the bulk of the streptomycin is produced concurrent with a rise in the pH of the fermentation and a marked decrease in mycelium weight. The lactic acid content remains relatively constant during this phase as does the soluble carbon content of the

TABLE 4. *Changes Characterizing the Two Phases During the Fermentation.*

	Phase I	Phase II
Streptomycin	Slight production	Maximum production
pH	Gradual rise	Reaches maximum
Mycelium	Rapid growth	Autolysis
Glucose	Rapid utilization	Small remaining amount exhausted
Soluble carbon	Gradual utilization	Reaches minimum and remains constant
Lactic acid	Slow production and utilization	Slow utilization
Oxygen demand	Maximum	Decreases to minimum
Soluble nitrogen	Used extensively	Concentration increases
Inorganic phosphorus	Used at maximum rate	Released into medium
Ammonia nitrogen	Rapid utilization	Concentration increases

medium. The oxygen demand slowly decreases during this phase. In general the oxygen demand seems to parallel the utilization of the glucose. During the second phase the ammonia nitrogen, soluble nitrogen, and inorganic phosphate content of the fermentation rise rather markedly paralleling the autolysis of the cells.

Illustrative data of these changes are presented in table 1. When vegetative inoculum has been used for the fermentation the growth phase has been markedly shortened, but the general changes appear to be the same on a given medium.

As mentioned before, the basal medium appeared to be low in inorganic phosphate and various quantities of potassium phosphate were added to supplement that already present. The most marked effect of this supplementation was on the streptomycin production which was decreased as indicated in table 2. Perhaps the buffering action of the added phosphate accounted for the slower rise in pH during the fermentation.

When the carbohydrate content of the medium was increased to 3 per

cent the growth phase was apparently quite drawn out as indicated by the data of table 3. The pH rise was very much slower during the fermentation of this medium. The rate of glucose utilization apparently remained at a high value until most of the fermentable carbohydrate was utilized as indicated by the prolonged moderate oxygen demand. Whereas the mycelium did not autolyze markedly until most of the fermentable carbohydrate had been utilized, it appears that the quantity of available carbohydrate did not determine the quantity of mycelium formed. As might be expected the release of the inorganic phosphate, increase in ammonia nitrogen and increase of soluble nitrogen (usually found during the autolytic phase) did not occur until late in the fermentation. The rate of production of streptomycin during the fermentation was much lower on this high carbohydrate medium. Apparently some autolysis of the mycelium occurs as the nitrogen content of the mycelium reaches a rather high value.

The utilization of the soluble carbon of this medium is not well understood. The accumulation of volatile acids during the fermentation could not be detected. Woodruff and Foster (1943) noticed that a considerable quantity of lactic acid is formed by *Streptomyces lavendulae* during the fermentation of various media. The strain of *S. griseus* used in the experiments reported here also forms lactic acid, but to a much lesser degree. This lactic acid is apparently the *dl* mixture as identified by the hydration and rotation of the zinc salt, although on this meat extract medium no doubt most of it came from the meat extract. When certain synthetic media are fermented by this organism considerable quantities of lactic acid are formed and this is also the *dl* mixture. Apparently certain strains of *S. griseus* may utilize lactic acid (this will be elucidated in a later publication) and the strain used in these experiments belongs in this group. These experiments indicate that the glucose is utilized mainly as an energy source and not for mycelium formation. The character of the unavailable soluble carbon compounds present during the second phase of the fermentation has not been determined. These come from the peptone and meat extract and are not synthetic products, since in synthetic media all of the soluble carbon (supplied as glucose) disappears from the medium.

The production of streptomycin seems to be associated with the autolytic phase of the fermentation. Whether this indicates that streptomycin is synthesized during the latter phase of the fermentation or is a constituent of the mycelium has not been clearly shown. The experiments of Waksman and others (1946) may support the latter hypothesis. Determination of proteinase during the fermentation indicates that during the second phase the extracellular pH 7 proteinase increases.[1] Although the effect of high

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[1] The proteinase determinations were made by following the hydrolysis of casein in a buffered medium by a method used by M. J. Johnson and associates.

phosphate in reducing streptomycin production might be attributed to toxicity, this concentration is no more than that present in cornsteep liquor media such as used by Schatz and his colleagues (1944).

SUMMARY

The chemical changes occurring during the fermentation of the Waksman-Schatz medium (1% glucose, 0.5% meat extract, 0.5% peptone, 0.5% sodium chloride) by a strain of *Streptomyces griseus* indicated that there are generally two phases of metabolic activity. During the growth phase the production of a mycelium is accompanied by a reduction in the soluble constituents of the medium (nitrogen, carbon, phosphorus), fermentation of the available carbohydrate, a high oxygen demand (Q_{O_2} may reach 150), and little production of streptomycin. During the autolytic phase the mycelium weight decreases markedly, inorganic phosphorus and soluble nitrogen are released into the medium, the oxygen demand drops, and considerable quantities of streptomycin are produced. The pH rises gradually throughout the fermentation. Increasing the carbohydrate content of the medium has little effect on the general changes although it prolongs the growth phase. Increasing the phosphate content has little effect on the general changes but reduces the quantity of streptomycin formed. Carbon dioxide apparently is the main product of glucose utilization for detailed investigation has revealed no large accumulation of volatile or non-volatile acids. Some lactic acid is formed during the fermentation; this substance is also utilized by this organism.

RAHWAY, NEW JERSEY

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SUPPLEMENTARY NOTES ON AMERICAN LABIATAE—IV

CARL EPLING

As material has accumulated through recent explorations, it has been my custom to supplement earlier revisions of American Labiatae by occasional notes describing new entities, giving noteworthy extensions of ranges, and modifying published keys to include new entities. These notes have previously appeared in this journal as follows: **67**: 509–536 (1940); **68**: 552–568 (1941); **71**: 484–497 (1944). The numbers preceding the specific names in the present paper are those used in the following appropriate revisions. **McClintock, E. & Epling, C.** A revision of *Teucrium* in the New World, with observations on its variation, geographical distribution and history. Brittonia **5**: 491–510. 1946. A review of the genus *Monarda* (Labiatae). Univ. Calif. Publ. Bot. **20**: 147–194. 1942. **Lewis, H.** A review of the genus *Trichostema*. Brittonia **5**: 276–303. 1945. **Epling, C.** Preliminary revision of American *Stachys*. Rep. Spec. Nov. Beih. **80**. 1934. The American species of *Scutellaria*. Univ. Calif. Publ. Bot. **20**: 1–146. 1942. Synopsis of the South American Labiatae. Rep. Spec. Nov. **85**: 1937. A revision of *Salvia*, subgenus *Calosphace*. Rep. Spec. Nov. **110**. 1939. A revision of *Hyptis* (ined.). **Epling, C. & Stewart, Wm. S.** A revision of *Hedeoma* with a review of allied genera. Rep. Spec. Nov. **115**. 1939.

TEUCRIUM

3. T. CUBENSE Jacq. subsp. LAEVIGATUM McClintock and Epling. TEXAS: Hidalgo County, 5 mi. s.w. of Weslaco, *Tidwell 38*.

MONARDA

8. M. LINDHEIMERI Engelm. and Gray. TEXAS: Gonzales County, Palmetto Park, *Albers 46099*.

11. M. PUNCTATA L. subsp. IMMACULATA Pennell. TEXAS: Bastrop County, Paige to McDade, *Tharp 32101*. Deep sand of the Carrizo in open woods, *Rodgers et al. 46529*.

12. M. FRUTICULOSA Epl. TEXAS: Kennedy County, Kennedy Ranch, *Tharp 42–40*.

14. M. CLINOPODIOIDES Gray. TEXAS: Travis County, Austin, *Warnock 245*. Hockley County, no locality, *Rachaner 132*.

17. M, CITRIODORA Cerv. TEXAS: Calhoun County, near Port Lavaca, *Gentry 45*. "Diminished form" referred to, p. 192.

TRICHOSTEMA

7. T. BRACHIATUM L. TEXAS: Walker County, 4 mi. e. of Huntsville, *Albers and Warnock*.

STACHYS

3. S. ERIANTHA Benth. ECUADOR: Azuay: Paramos, vicinity of Toreador, 3810–3930 m., *Steyermark 53178.*

S. ? HAMATA Epl. VENEZUELA: Sucre: Cerro Turumquire, north-facing slopes between La Trinidad and zone of cloud forest, 1700–2000 m. *Steyermark 62510.* Very similar to this species, known only from the Paramo de Quindio, Colombia, but more lax and the trichomes elongate and scarcely pustulate at the base. In many respects resembles *S. nubilorum* Epl. of Guatemala.

SCUTELLARIA

6. S. AUSTRALIS (Fassett) Epl. TEXAS: Lavaca County, 3½ mi. n.e. of Hallettsville, *Webster 2013B.* Tyler County, 8 mi. w. of Woodville, *Rowell et al. 47213.*

26. S. WRIGHTII Gray. TEXAS: Williamson County, without locality, *Dyksterhuis et al. 16110.*

33. S. DRUMMONDII Benth. TEXAS: Kendall County, Edge Falls, *Tharp et al. 17T160.* Lee County, 1 mi. s. of Lincoln, *Rowell et al. 17M097.*

100. S. SPECIOSA Epl. What seems to be this species has been collected in PERU: Cuzco: Quispicanchi, Mandor; Marcapata, 1200 m., *Vargas 3811.*

102. S. ATRIPLICIFOLIA Benth. ECUADOR: Pichincha; wet luxuriant forested slopes or barrancos between kms. 37 and 50, along Rio Saloya, 1830–2430 m., *Steyermark 52534.*

106. S. VOLUBILIS Kunth. ECUADOR: Azuay: dry rocky slopes bordering Rio Leon towards Oña, 1970 m., *Steyermark 53701.*

109. S. BENTHAMIANA (Mansf.) Epl. PERU: Cuzco: Paruro, Huanoquite, *Vargas 2342.*

SATUREJA

S. LINEATA Epl. ECUADOR: Azuay: between kilometer 67 and south towards Oña, 3715 m., *Steyermark 53668.*

POLIOMINTHA

3. P. GLABRESCENS Gray. TEXAS: Presidio County, in creek bed in loose soil, Burnt Camp Canyon, the Solitario, 3800 ft., 30.XI.41, *Hinckley 2261.*

HEDEOMA

2a. **H. subaequale** Epl., sp. nov. Fruticulus altitudine ad 30 cm. ramulis gracilibus retrorse hirtellis; foliorum laminis ascendentibus anguste ellipticis etiam sublinearibus, 6–14 mm. longis, 1.5–3 mm. latis, in apice obtusiusculis sessilibus, integris, utrimque punctatis, glabris; cymulis plerumque 1-floribus; pedicellis gracillimis 6–9 mm. longis; calycum tubo 4.5–5 mm. longo, extus glabro, dentibus omnibus erectis anguste lanceolatis, acutissimis, 2.5–3 mm. longis, similibus, superioribus tamen quam inferiores paulo brevioribus, annulo sat denso; corollarum purpurearum tubo circiter 12 mm. longo intus pilis minutis crassiusculis sparse hirtello, vix tamen annulato.

MEXICO: San Luis Potosí. Rocky limestone, mountain above San Pedro, 2150–2200 m., 29.VII.1934, *Pennell 17731* (UCLA). Prope Morales in convalle San Luis Potosí, X.1876, *Schaffner.* San Luis Potosí, 1879, *Schaffner 412; 352.*

Allied to *H. apiculatum* Stewart, known only from the Guadalupe Moun-

tains of New Mexico and Texas (*Goodding 6023, Moore & Steyermark 3563*), from which it differs primarily in the conformation of the calyx and calyx teeth. This fruticulose section of *Hedeoma,* including also *H. hyssopifolium,* leans strongly toward the genus *Poliomintha,* from which it may be distinguished by the exannulate corolla tube.

7. H. MANDONIANUM Wedd. PERU: Cuzco: Paruro, Sullcan, Ayusbamba, 4020 m., *Vargas 887.*

16. H. REVERCHONI Gray. TEXAS: Llano County, near Enchanted Rock, *Barkley et al. 47307.*

17. H. ACINOIDES Scheele. TEXAS: Lee County, 1 mi. s. of Lincoln, *Bailey et al. 17M098.*

23. H. COSTATUM Gray. MEXICO: Hidalgo: 30 mi. n. of Zimapan in oak woodland, *Barkley 17M153.*

SALVIA

10. S. BALLOTAEFLORA Benth. MEXICO: Tamaulipas: 20 mi. west of Reynosa, *Painter and Barkley 14392.*

21a. **S. Vargasii** Epl., sp. nov. (Tomentellarum) per specim. in Peruviae prov Cuzco prope Kairancka a Vargas (no. 5834) lectum constituta est; typus in herb. Univ. Calif. (Los Angeles).

Frutex sarmentosus altitudine ad 1 m. ramulis gracilibus minutissime hirtellis et glandulis sessilibus aureis conspersis internodiis quam folia plerumque brevioribus; foliorum laminis maximam partem 1–1.5 cm. longis, sat tenuibus, anguste ovatis, in apice obtusiusculis, in basi plus minusve truncatis, marginibus serratis, pagina superiore sparse hirtella, inferiore glandulis sessilibus aureis obtecta, petiolis gracilibus 3–4 mm. longis elatis; floribus paucis oppositis in racemis 2–3 cm. longis dispositis, bracteis caducis; calycibus florentibus 6 mm. longis, in maturitate paulo auctis, extus sparse hirtellis et glandulis sessilibus aureis conspersis, labia superiore curta mucronata; corollarum cyanearum tubo 14 mm. longo; staminibus subintegris, stylo minute hirtello fere glabro.

PERU: Cuzco: Grau: prope Kairancka, 2400 m., in declivibus aridis inter fructices, 9.III.1946, *Vargas 5834.*

Very similar indeed in aspect to *S. fruticulosa* of Oaxaca, but with notably larger flowers. At the same time its foliage recalls that of *S. Gillicsii* of the southern Andes, but much diminished. The stamens are almost entire, hence in the key to the sections it might be sought in *Flocculosae.*

57. S. CORRUGATA Vahl. ECUADOR: Azuay: between Milleturo and Toreador, 2590–3900 m., *Steyermark 53026.* Between km. 67 and south towards Oña, 3715 m., *Steyermark 53673.*

145. S. HIRTA Kunth. ECUADOR: Azuay: dry rocky slopes bordering Rio Leon towards Oña, 1970 m., *Steyermark 53708.*

160. S. UNCINATA Urb. SANTO DOMINGO: San Juan: Sabana Nueva, 6200 ft., cordillera central, n. of Rio Arriba del Norte, *R. A. and E. S. Howard 9155.* Apparently this, but leaves lanceolate and elongate; no mature flowers.

164. S. THORMANNI Urb. SANTO DOMINGO: San Juan: pine woodlands, Piedra del Aguacote to Rio del Oro, 5200 ft., *R. A. and E. S. Howard 9382.* Apparently this, but no flowers. Said to be vine-like.

167. S. COCCINEA Juss. TEXAS: Brownsville, *Shiller 864; 637.* A color variant in which the corollas appear to be of a more bluish red. *Fisher 41034.*

201. S. (?) SARMENTOSA Epl. Peru. Apurimac. Grau: Cuesta de Seccsecka in declivibus inter frutices, 3800 m., 4.III.1946, *Vargas 5763*. Vargas' specimen is very similar to one gathered by Stafford in Urcos valley, Cuzco, save only in the notably larger flowers (calyces 11 mm. long, larger at maturity; corolla tube 12 mm. long). I previously referred the latter to *S. sarmentosa*, described from Ollantaytambo, despite differences in pubescence and habit. It is difficult to say whether two species are actually represented. In any case, both forms find their closest alliance with *S. lycioides* and *S. Greggii* of northeastern Mexico.

204. S. LYCIOIDES Gray. TEXAS: Presidio County, Chinati Mts., about 10 mi. w. of Shafter, *Hinckley 3538*.

226. S. SPHACELIFOLIA Epl. MEXICO: Sinaloa: Sierra Surotato, Canyon de Tarahumare, 3000–4000 ft., mixed subtropical vegetation with lower oaks. *Gentry 7295*. Sierra Surotato, Los Pucheros, 5500–6500 ft., pine-oak-madrone, open forested slope. *Gentry 7209*.

239. S. AMISSA Epl. ARIZONA: Graham County, on rocky shaded canyon slope; 3200 ft. elev.; 10 mi. n.w. of Klondyke in Aravaipa Canyon. *Darrow*.

242. S. FARINACEA Benth. TEXAS: Shelby County, 12 mi. n.w. of Center, *Lee 101*. McLennan County, near Waco, *York 16143*.

249. S. MALACOPHYLLA Benth. ECUADOR: Loja: between S. Pedro and Chinchas, 1600 m., *Espinosa 1297*. Originally described from Mathew's collection near Sesuya, Peru and not since collected. *S. lorensis* known only from Hartweg's collection near Loja is similar but glabrate. It may be only a variant. If so the latter name has priority, but both may be conspecific with *S. mitis* R. and P.

305. S. TILIAEFOLIA Vahl. TEXAS: Presidio County, street in Marfa, 17.X.1942. *Hinckley 2636*. First record for the United States. Arizona. Cochise County, deep shade of limestone cliff, 5500 ft., Mustang Mts., near Harrison Ranch, *Darrow 3628;* apparently indigenous, according to the collector.

306. S. OBVALLATA Epl. COLOMBIA: Cauca: Cord. Central vertiente occ. Hoya del rio Palo, matorrales y bosques subseriales en La Tolda, arrica de Tacueyo, 2000–2030 m. 18.XII.L944. *Cuatrecasas 19409*.

344. S. (?) AMPELOPHYLLA Epl. VENEZUELA: Merida: Paramo de los Colorados, between El Molino and San Isidro Alto, 2745–2955 m., *Steyermark 56540*. Moist rich woods, Los Quebraditos, above Juji, 2509 m., *Steyermark 55997*.

This species is known from several collections in El Cauca. Whether the present collections are referrable to it is uncertain. The flowers are smaller (corolla tube 12 m. long), pale blue or white, and the corolla tube is not invaginate. Yet the habit and aspect of the rather poor material is remarkably similar. In the key to the sections, these specimens would be sought under section *Angulatae*, because of the short tube.

365a (177). S. ORBIGNAEI Benth. Recent collections by Cardenas permit an emendation of the characters of this species. The stamens are included within the upper lip, and not exserted, as indicated in the sectional description. In pressing, they frequently become extruded and give that appearance. The lower lip is shorter than the upper, or at most subequal. Although the base of the corolla tube constantly bears two well defined papillae within, the degree of invagination is variable. White-flowered forms may occur accord-

ing to Cardenas. For these reasons, it seems desirable to assign *S. Orbignaei* to sect. *Pavonia* as no. 365a, amending that section to include simple as well as branched hairs, since both may occur in *S. Orbignaei*, and to assign *S. perlonga* to a new section, *Nelsonia* (see below). The amended key to the section *Pavonia* would read as follows:

Corollarum tubi 13–14 mm. longi.   364. *S. revoluta.*
Corollarum tube 19–24 mm. long.
   Folia in basi truncato-subcordata; plantae peruvianae.   365. *S. Macbridii.*
   Folia in basi angustata; plantae bolivianae.   365a. *S. Orbignaei.*

Following are Cardenas' collections: BOLIVIA: Cochabamba: Morochata, 2900 m., *Cardenas 2497;* Angostura, 2560 m., *Cardenas 2396;* Orami, 2700 m., *Cardenas 2395;* Ansaldo, 3000 m., *Cardenas 2447*.

39. Nelsonia Epl., sect. nov.

Suffrutices foliis oblongis rugosis; calycum labia superiore 5-venis paulo longiore; corollarum coccinearum vel alarum sat crasso ventricoso ad basim constricto-invaginato et intus rugis binis obliquis ornato, labia superiore quam inferior longiore; staminibus e galea 3–4 mm. exsertis, ad fauces positis; jugo ad connexum in dentem parvum producto; styli villosi ramo postico longiore. Plantae Mexicanae; species typica est *S. perlonga*. The key to the sections should be amended to read as follows:

p. 6 line 7: "EE. Corollarum tubi fere recti ad basim sub papillas invaginati; stamina ad fauces posita. 39. *Nelsonia.*"

p. 11 line 31: "I. Pili ramosi. J. Plantae peruvianae vel bolivianae; corollarum tubi 13–24 mm. longi. 64. *Pavonia* (vide etiam *Cardinales*)."

p. 12 line 6: "L. Corollae ad basim valde invaginatae nullomodo lateraliter saccatae. M. Folia ampla in basi rotundata vel cordata; plantae brasilianae. 76. *Hoehneana.* MM. Folia oblonga in basi angustata. 64. *Pavonia.* LL. Corollae ad basim ———."

368a. **S. iuliana** Epl., sp. nov. (65. *Nobiles*)

Herba perennis ramosa altitudine ad 2 m., ramis hirtellis, foliorum laminis membranaceis 5–6 cm. longis, 2–2.5 cm. latis, ellipticis utrimque acuminatis, marginibus serratis, paginis ambabus glabris nisi sparsissime hirtellis, petiolis ad 1 cm. longis elatis; floribus (in specimini suppetenti) in foliorum axillis solitariis, pedicellis gracilibus ad 15 mm. longis elatis; calycibus florentibus circiter 22 mm. longis, in maturitate paulo auctis, labiis acuminatis subaequilongis 8–9 mm. longis superiore sub-5-venis, inferiorum dentibus omnino connatis; corollarum coccinearum tubo circiter 30 mm. longo, ad basim paulo constricto, labia superiore circiter 18 mm. alto, inferiore breviore. VENEZUELA: Merida: Cuesta de Rincon and Cuesta del Barro, between Canagua and La Quebrada, on road to El Molino, 1925–2175 m., May 10, 1944, *Steyermark 56450* (type, U.C.L.A.).

The large-flowered sections *Nobiles, Longiflorae,* and *Tubiflorae* are closely allied. The two former are separable chiefly by the habit of the foliage, the latter is distinguished by the usually 3-veined upper calyx lip. Steyermark's accession appears to be more nearly allied to *Nobiles* and is similar in many ways to *S. balaustina* of east central Brazil.

420. S. LATENS Benth. VENEZUELA: Merida: dry rocky gneissic-granitic slopes between Timotes and Paramite, 2285–3500 m., *Steyermark 55700.* Apparently this, known previously only from Colombia.

443d. S. anguicoma Epl., sp. nov. (87. *Purpureae*). Herba suffruticosa altitudine ad 2 m., ubique pilis crassioribus anguicoma; foliorum laminis ovato-ellipticis 5–7 cm. longis, 2.5–3.5 cm. latis, in apice breviter acuminatis, in basi ad petiolos saepius 2–4 cm. longos cuneato-angustatis, marginibus serratis, venis subtus prominulis; floribus plerumque tribus in verticillastris; calycum florentium tubo 5–6 mm. longo, labiis subaequilongis, dentibus acuminatis; corollarum purpurearum tubo circiter 12 mm. longo. VENEZUELA: Merida: Woods above Las Cuadras, 1520–2285 m., March 30, 1944, *Steyermark 55830*.

Following is a revised key to *Purpureae* (p. 329):

Plantae colombiano-venezuelanae.
  Folia utrimque pilis crassioribus villoso-anguicoma.   443d. *S. anguicoma.*
  Folia subtus molliter vel tomentosa vel pubescentia, interdum glabrata.
    Corollarum tubi plerumque 10–12 mm. longi.
      Ramuli molliter tomentosi; calyces lanata.   443. *S. sordida.*
      Ramuli plus minusve villosi; calyces glabrata.   443c. *S. Cuatrecasana.*
    Corollarum tubi 15–16 mm. longi.
      Calyces florentes 7.5–8 mm. longi; folia subtus molliter pubescentia.
                   444. *S. rufula.*
      Calyces florentes 9–10 mm. longi; folia subtus dense molliterque
        tomentosa.   445. *S. tolimensis.*
Plantae boreali-americanae.
  Folia subtus solum ad venas pilis crassioribus villosa; calyces 11 mm.
    longi.   441a. *S. Matudae.*
  Folia subtus pilis gracilibus modo pubescentia vel tomentosa modo glabrata; calyces 4–8 mm. longi.
    Petioli plerumque 2–4 cm. longi et ultra, folia in basi plerumque rotundata.
      Corollae extus pilis crassis dense villosae; tubi fauces 3–6 mm.
        diametro.   441. *S. Littae.*
      Corollae extus pilis tenuibus pubescentes; tubi fauces 1.5–3 mm.
        rarius 4 mm. diametro.   442. *S. purpurea.*
    Petioli plerumque 1–1.5 cm. longi.
      Corollarum tubi 18–22 mm. longi.   440. *S. curviflora.*
      Corollarum tubi 10–12 mm. longi.
        Folia subtus tomentosa, superne areolato-hirsuta.   443a. *S. areolata.*
        Folia utrimque praesertim ad venas hirtella.   443b. *S. sparsiflora.*

HYPTIS

1a. H. cuniloides Epl., sp. nov. (1. *Umbellaria*) Fruticulus altitudine circiter 20 m., ramulis incano-puberulis; foliorum laminis fasciculatis 2–3 mm. longis, plerumque obovatis, integris sessilibus, pilis minutissimis argenteis; cymulis 3-floribus in foliorum supremorum pedunculis 5–6 mm. longis elatis; calycibus florentibus glanduloso-villosulis, in maturitate 4 mm. longis, dentibus deltoideo-subulatis vix 1 mm. longis; corollarum tubo circiter 6 mm. longo, superne sat ampliato; gynobasi non viso; nuculis ut videtur laevibus. BRAZIL: Bahia: Mun. Morro do Chapeu, sandy soil, treeless "subalpine" zone, 1000 m., with *Velloziaceae* etc., April 1944, *Schery 587* (type, Mo. Bot. Gard.).

Resembles *Cunila incana*. Apparently referable to section *Umbellaria*, but differs markedly in aspect, perhaps because of the minute leaves. The character of the nutlets and gynobase could not be clearly discerned.

59. H. FLORIBUNDA Briq. ARGENTINA: Santa Fe: Lauteri, depto. Gral, Obligado, *Maldonado 1716*.

142. H. LACINIATA Benth. COLOMBIA: Meta: edge of thicket, 200 m., sandy flood plain of Rio Muco, 70 km. s.e. of San Pedro de Arimena, *Hermann 10932*.

143. H. ULIGINOSA St.-Hil. BRAZIL: Rio Grande do Sul: San Salvador, *Eugenio Leite 751*.

147. H. MICROPHYLLA Pohl. VENEZUELA: Bolivar: Tumeremo, 305 m., *Steyermark 60923*.

269. H. OBTUSIFLORA Presl. ECUADOR: Loja: Torata, *Espinosa 1190*.

DEPARTMENT OF BOTANY, UNIVERSITY OF CALIFORNIA

LOS ANGELES, CALIFORNIA

TORREYA

FIELD TRIP REPORTS

MAY 11. PERKIOMEN VALLEY, MONTGOMERY COUNTY, PA. This was a joint outing through the courtesy of the Academy of Natural Sciences of Philadelphia. Torrey Botanical Club members were a distinct minority but none the less interested and appreciative. The outing included a stop at Kibblehouse Quarry, Perkiomenville, where the geology was explained and identification of various rocks was made by Dr. E. T. Wherry. Nice stands of *Viola striata* and *Mertensia virginica* were discovered along Perkiomen Creek adjacent to the quarry. A further fine display of spring flowers was found along Unami Creek near Sumneytown, most noteworthy being *Cercis canadensis, Viola triloba, V. conspersa, Castilleja coccinea, Aquilegia canadensis,* and *Caltha palustris.* Total attendance 78. Leader for Torrey Botanical Club, Louis E. Hand.

MAY 30–JUNE 1. TANNERSVILLE, PA., in the Poconos. Participation in this weekend was also through courtesy of the Academy of Natural Sciences of Philadelphia. The program opened with a trip to Big Pocono Mountain. Enroute, our guide from the Pennsylvania Department of Forestry showed the group what is considered the largest *Tilia americana* in the United States. It measures 20 ft. 7 in. in circumference at the ground, 16 ft. 3 in. at breast height, is 104 ft. 9 in. tall, and has a spread of 75 ft. Its age is estimated at 350 years. The summit of Big Pocono (2,131 ft.) afforded excellent views but little difference in vegetation from the Tannersville region. *Rhododendron roseum* Rehder, the northern, mountain relative of our coastal plain *Rhododendron nudiflorum,* and called *R. canescens* in Gray's Manual, was the outstanding floral attraction. It was found in abundance and at the height of bloom. Other trips included a fossil coral reef at Analomink, the vicinity of Buck Hill Falls Inn, Tannersville bog, and Swiftwater Falls. Plants of northern affinity encountered included *Kalmia polifolia, Andromeda glaucophylla, Picea mariana, P. rubra, Larix laricina, Eriophorum callitrix, Vaccinium Oxycoccus, Carex limosa, Calla palustris,* and *Fraxinus nigra.* Attendance over 100 (13 Torrey Botanical Club members). Leader for the Club, Louis E. Hand.

JUNE 14–15. DELAWARE WATER GAP, PENNA.–N. J. Saturday was devoted to a hike along Kittatinny Mtn. and over Mt. Tammany from where panoramic views from the rocky outlook were enjoyed by all. A botanical survey list was compiled for this newly opened section of the Appalachian Trail. The Sunday hike was also along the A. T., ascending Mt. Minsi on the Penna. side of the gap. General opinion was that views from Tammany excel. Attendance 9. Leader, Louis E. Hand.

JUNE 15. MILLBURN, N. J. "This was a pleasant little trip through the various paths and bridle paths of Sounth Mountain Reservation where many of our common flowers were noted, particularly *Krigia amplexicaulis* and *K. virginica,* the former abundant throughout and the latter in a fair stand on rocks. The highlight of the trip, botanically, was the 'Blazing Star,' *Chamaelirium luteum* (L.) Gray which is not recorded in Essex County, N. J. by Norman Taylor's Flora of the Vicinity of New York." Attendance 10. Leader, William Rissanen.

JUNE 21–22. FORKED RIVER, N. J. Many new species were added to our list from this locality. The most spectacular find was *Listera australis,* of which a stand of eight plants was found. One specimen was taken for deposit in the herbarium of the Academy of Natural Sciences at Philadelphia where the other known specimens from N. J. are deposited. Something of a record may also have been set by David Fables who drove from the Gaspe directly to Forked River for this trip. Attendance 9. Leaders, Louis Hand and Hollis Koster.

JUNE 22. ARDEN CIRCULAR IN PALISADES INTERSTATE PARK. A small group with much energy lengthened the walk somewhat. Plants reported to be of interest at the time were *Rhododencron maximum* (apparently nearly a month from flowering), *Calla palustris*, and *Sarracenia purpurea*. Attendance 3. Leader, Dr. H. B. Gordon.

JULY 12. WEST ORANGE, N. J. Approximately 100 species and varieties of ferns and fern allies were growing in the garden of our leader at this time. They attracted much attention and favorable comment. Attendance 18. Leader, W. H. Dole.

JULY 13. HAMDEN, CONNECTITUT. Torrey Botanical Club people were again in the minority, this being a joint outing with the Connecticut Botanical Society. Those present expressed much interest in the chestnut plantations. Attendance 40. Leader, Dr. A. H. Graves.

JULY 19. WARD'S POINT, STATEN ISLAND, N. Y. "We were glad to note that the two local celebrities in the botanical line were still there. *Allionia nyctaginea* and *Polanisia trachysperma* seem to hold their own. In addition we found a third rather rare adventive: *Lythrum alatum*. I found a single plant of this a few years ago but now there was a colony of several dozen. Among the more usual plants worth mention were *Asclepias tuberosa*, fairly abundant here though it is said to be becoming rare on the Island. Several Euphorbias grow here including *E. Cyparissias* and *E. polygonifolia* as well as the ubiquitous *E. maculata*. *Convolvulus sepium* and *C. arvensis* were in evidence also. Among the trees of interest were *Morus alba* with fruit in just the right condition for sampling, *Populus alba*, and *Salix alba*. *Broussonetia papyrifera*, and *Celtis occidentalis*." Attendance 12. Leader Mr. Charles Ericson.

AUGUST 2–3. MONTAUK POINT, LONG ISLAND, N. Y. "We were especially honored and happy to have with us Dr. John H. Barnhart, in point of years of membership, the oldest living member of the Torrey Botanical Club. His genial presence added much to the enjoyment of the trip. We were also fortunate in having Mr. E. J. Alexander with us, who showed us many extremely interesting and rare plants which we would otherwise have missed, and who checked our identifications." Attendance 23. Leader Dr. H. N. Moldenke.

AUGUST 10. QUARRY LAKE, VALHALLA, N. Y. This trip, through the courtesy of Mr. Nathan Straus, included a tour of the estate and study of the many interesting plantings. Attendance 15. Leaders Mr. Ernest Hoelle and Dr. Harold N. Moldenke.

AUGUST 24. SEELEY'S NOTCH, SCOTCH PLAINS, N. J. This walk through the Watchung Mountains provided a great number of the plants to be expected in such terrain, and lists of these have been published in seasons past. Newark Museum Nature Club members and friends were our guests. Attendance 23. Leader, Dr. Harold N. Moldenke.

AUGUST 20–29. MONTREAL AND THE GASPÉ REGION, QUEBEC. Joint affair with the Botanical Society of America and the American Society of Plant Taxonomists in connection with the establishment of a Northeastern Section of the Botanical Society of America. The first two days were given over to lectures, symposia, and miscellaneous papers together with exploration of the remarkable Jardin Botanique. On Friday a trip to Morgan's Woods gave an intimate view of the deciduous forest as represented north of Montreal. A carefully prepared and fully documented tour guide for the Quebec-Gaspé excursion arranged by Dr. Pierre Dansereau and Mr. Marcel Raymond, was fully appreciated as the days ensued. Since this is to be revised and made available generally, the present report will note only the places visited. A surprisingly large percentage of the characteristic species listed in the tour guide were seen at each stop by those who looked carefully. Many additional species were turned up in the process of combing the various localities and referred to the leaders for identification or confirmation. The presence of Mr. Ernest Rouleau, Curator of the Marie Victorin Herbarium of the Institute Botanique of the University of Montreal was an added reassurance. Places visited and their objectives follow: Cap-de-la-Madeleine, pine barrens (substitute *P. rigida* for *P. banksiana* and one would think himself back in Jersey, for the most part). Sainte-Anne-de-la-Perade, Typical St. Lawrence lowland forest vegetation. Neuville, fresh-water intertidal zone (shingle beach and ledges).

Duchesnay, deciduous forest and coniferous forest more or less intermixed (overnight at Forest Rangers School). Saint-Vallier, deciduous forest, intertidal zone, tidal pools and other estuarian elements but not fully salt water. Sainte-Anne-de-la-Pocatiere, grounds and adjacent forest of a school of agriculture. Kamouraska, sufficient stops in the vicinity to impress the geological and botanical formations of this name upon everybody, particular attention given to the marshes and their zonation. Saint-Fabien, raised bog (with an extra one thrown in en route). Le Bic, salt marsh, silty intertidal area, and rocks. Baie-des-Sables, shore zonation (*Mertensia maritima* and *Senecio Pseudo-Arnica* got most of the attention). Sainte-Anne-des-Monts, the wonderful sequence of forest, scrub, alpine meadow, ravines, and stony summit of Mt. Albert is beyond description. Mont-Saint-Pierre, plants of the shifting schists. Perce, endemic and other plants of exposed cliffs and crevices together with the more extensive communities of grass and woodland. Paspebiac, sand bar vegetation. Bonaventure River flats, just that but with some nice endemics and more widely spread species. Salt marshes were also studied here. Attendance, over 100 with 80 odd making the tour in 23 cars. Leader, Dr. Pierre Dansereau.

INDEX TO AMERICAN BOTANICAL LITERATURE

COMPILED BY

LAZELLA SCHWARTEN

WITH THE COLLABORATION OF THE EDITOR OF THE TAXONOMIC INDEX

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